The efficiency of cyproconazole and fludioxonil for plant protection against the phytopathogenic fungus Botrytis cinerea

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Abstract. The effect of two active ingredients of fungicides on pure culture of Botrytis cinerea Pers was assessed. The analysis was carried out for 16 possible combinations of cyproconazole and fludioxonil, one of which was a control one. According to the results of measurements of the dry weight of mycelium in an accumulative culture, a diagram was constructed and two-factor analysis of variance was given. It has been established that the combined effect of these fungicides has a significant effect on the growth of mycelium. The following combinations had significant effect: fludioxonil at maximum concentration (0:10), 1 part of cyproconazole and 10 parts of fludioxonil (1:10), 5 parts of cyproconazole and parts of 10 fludioxonil (5:10), 10 parts of cyproconazole and 5 parts of fludioxonil (10:5), and the maximum concentrations of two substances (10:10).

1. Introduction
Phytopathogenic fungi cause damage to agriculture. The use of fungicides is a targeted event to protect plants from disease. The use of modern means can significantly reduce crop losses. It is important to use fungicides safely and timely [2]. The development of new products is aimed at efficiency of active substances; preparation of the composition of several substances in such a way as to achieve the effect of mutual enhancement of action; selection of optimal doses of exposure. In addition, the objectives of cost reduction, composition optimization, and others are pursued.

Species of the genus Botrytis are a convenient model for research the action and efficiency of fungicides. In addition, they are characterized by an extremely wide range of affected plants from different families (Solanaceae, Fabaceae, Apiaceae, Brassicaceae, and Asteraceae). The disease, grey rot, affects more than 200 dicotyledonous plant species and a few monocotyledonous plants found in temperate and humid regions [14]. Serious economic losses can be a result of this disease to both field and greenhouse grown crops. The causal agent, Botrytis cinerea can infect mature or senescent tissues, plants prior to harvest, or seedlings. There is a wide variety of hosts infected by this pathogen.
including protein crops, fiber crops, oil crops, and horticultural crops. Horticultural crops include vegetables (examples are chickpeas, lettuce, broccoli, and beans) and small fruit crops (examples are grape, strawberry, and raspberry), these are most severely affected and devastated by gray mold [14]. Plant organs affected include fruits, flowers, leaves, storage organs, and shoots. Grey rot can be chemically controlled with well-timed fungicide applications starting during the first bloom. Timing can reduce the chance of resistance and will save on costs [13]. Objective of the research was to assess the efficiency of the joint application of the active ingredients of fungicides cyproconazole and fludioxonil, which are included in the preparation KingCombi (Agro Expert Group LLC) at concentrations of 34 g l⁻¹ and 8.3 g l⁻¹, respectively.

Fludioxonil is a non-systemic fungicide, introduced in 1993 by Ciba-Geigy (now Syngenta). It is used for the treatment crops (particularly cereals, fruits and vegetables, and ornamental plants; often in combination with another fungicide such as cyprodinil). Fludioxonil is used against Fusarium, Rhizoctonia, Alternaria and Botrytis cinerea [11]. It controls the following pests: Alternaria, Ascochyta, Aspergillus, Fusarium, Helminthosporium, Rhizoctonia and Penicillium spp., Tilletia [3]. It is toxic to fish and other aquatic organisms [11]. Its mode of action is to inhibit transport-associated phosphorylation of glucose, which reduces mycelial growth rate [12].

Cyproconazole is an agricultural fungicide of the class of azoles, used on cereal crops, coffee, sugar beet, fruit trees and grapes, [8] on sod farms and golf courses and on wood as a preservative [4]. It was introduced to the market by then Sandoz in 1994 (which is Syngenta as of 2000). Cyproconazole inhibits demethylation, a particular step in the synthesis of a component of the fungal cell wall called sterol. This means it affects fungal growth, but not the fungal sporulation. This explains why it must be used when fungal growth is maximum, early in the infection, because in late infections fungal growth slows down and the agent is ineffective [5]. Cyproconazole is used against powdery mildew, rust on cereals and apple scab, and applied by air or on the ground to cereal crops, coffee, sugar beet, fruit trees and grapes [8]. It controls the following pests: Puccinia graminis, Puccinia spp., Pseudocercosporella herpotrichoides and Septoria species [6]. Also it inhibits Septoria, Pyrenophora, Fusarium, Ustilago, Rhynchosporium, Bipolaris, Cercospora [7].

2. Materials and methods
The object of the study was the fungus Botrytis cinerea Pers., Fungi, Ascomycota, Pezizomycotina, Leotiomycetes, Leotiomycetidae, Helotiales, Sclerotiniaceae, Botrytis [10]. Strain MFG58984 was provided by the laboratory of mycology and phytopathology of All-Russian institute of plant protection (FSBSI VIZR), St. Petersburg. The strain was cultivated on potato-sucrose agar with erythromycin lamp. We used selective light with a wavelength of 350 - 370 nm, an intensity of 70-150 μmol·cm⁻² for induction of sporulation.

Fungicide solutions were prepared by dilution of working solution with a concentration of 5 mg·ml⁻¹. The fungicides were pre-dissolved in 500 μl of cyclohexanone, and then the emulsion was prepared by dilution of sterile water. The working solution was added into sterile flasks with 50 ml of Chapek’s sterile medium with the addition of thiamine and biotin. The concentrations of cyproconazole and fludioxonil were respectively 0; 0.0; 1.0; 5.0; 10.0; 1; 0.1; 1; 1.5; 1:10; 5:0; 5:0; 1:5; 1:5:10; 10:0; 10:1; 5; 5:10:15 ppm; 16 variants in total. Then 100 μl of a suspension of conidia of fungus B. cinerea which contains 10⁷ CFU was added to the flasks. The fungus cultures were grown in a sterile box at a temperature of 22° C for 12 days. At the end of the incubation, the contents of flasks were filtered through paper filters “white ribbon”, dried at 55° C to constant weight. Then the filters with culture were dried at 55° C to constant weight and weighed again on analytical scales.

There were two replications. The arithmetic means, standard errors of means, and Student-t criteria were calculated, significance of means were determined at P = 0.05. The results are shown in figure 1. Two-factor analysis of variance was carried out according to the obtained data [9]. The results are shown in table 1.
3. Results and discussion
Influence of cyproconazole and fludioxonil on plants and diseases was demonstrated earlier [15, 16]. Study of the effect of fungicides directly on the phytopathogenic fungus was of interest.
Fludioxonil are shown in figure 1 to decrease the growth of mycelium with increasing concentrations (0: 1, 0:5, 0:10). Such a pattern is not typical for cyproconazole (1:0, 5:0, 10:0).

![Figure 1. Dependence of the dry weight of mycelium on the concentration of fungicides.](image)

A phenomenon of increasing of the dry weight of mycelium under the influence of small doses of fungicides (1 ppm) was demonstrated. Visually, the growth of mycelium was not noticeable, but a significant amount of transparent mucus was formed in the flasks, which can indicate a protective reaction of the fungus. In the future, it is necessary to conduct a thorough study of the chemical composition of biomass of fungus. The dry weight of mycelium from a flask with cyproconazole and fludioxonil with ratio of 1:1 weight was greatest; it significantly exceeds the control (0:0).

The minimum dose of fludioxonil also increased the growth of mycelium. The weight of micelium exceeded control (0:1). The weight of mycelium exceeded the same in control variant (0:0) when fungicides in ratio (cyproconazole: fludioxonil) 5:0, 5:1, 10:0 ppm were taken. The minimum dose of cyproconazole inhibits the growth of mycelium. The most effective inhibiting ratios of cyproconazole: fludioxonil were 0:10, 1:10, 5:10, 10:5, 10:10.

| Table 1. Results of two-factor analysis of variance of dry weight of mycelium under the influence of cyproconazole and fludioxonil. |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|
| Indicators                        | Formula         | Considered effect of factors | Unconsidered factors |
| sum of squared deviations         | $S^2_t$         |       444.00     |       63.84     |       742.07     |       251.20     |       1,501.10 |
| degree of influence of factors    | $\eta^2_t = \frac{S^2_t}{S^2_y}$ | 0.30  | 0.04  | 0.49  | 0.17  | - |
| number of degrees of freedom dispersions | $\nu_t$ | 3    | 3    | 9    | 16    | - |
|                            | $S^2_t$         |       148.00    |       21.28    |       82.45    |       15.70    | - |
| Fisher criterion                | $F = \frac{\nu_t S^2_t}{S^2_y}$ | 9.43 | 1.36 | 5.25 | - | - |
According to two-factor analysis of variance (table 1), cyproconazole has significant influence on the growth of mycelium. The degree of influence of cyproconazole was 0.30 in this experiment. Due to the effect of stimulation the degree of influence of fludioxonil was extremely low, 0.04. The combined effect of cyproconazole and fludioxonil exceeds the critical value; the degree of influence on the growth mycelium was 0.49. It was more than the sum of 0.30 and 0.04. Consequently, the fungicides have a synergistic effect on the growth of *B. cinerea* under laboratory conditions.

4. Conclusion

Thus, the combined application of both fludioxonil and cyproconazole in minimal concentrations had a stimulating effect on the growth of the mycelium of *B. cinerea*. In addition, the amount of mucus produced by the fungus has increased. This can indicate a defensive reaction of the fungus. The application of only cyproconazole even at high doses was not effective enough to inhibit the growth of mycelium of *B. cinerea*. Despite the high fungistatic efficiency of fungicides in high doses, it is necessary to remember about the possible toxic effect of fungicides on the plants as well as toxigenic effect of fungicides on the fungus [1]. Therefore, the development of a fungicide requires not only research with pure cultures, but also experiments on vegetative plants.

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