

New fungicides for protecting vines infected with downy mildew

Viktor Dolzhenko¹, Olga Kungurtseva^{1,2}, Maria Revkova^{1*}, Yevgenia Yurchenko³, and Natalia Aleynikova⁴

¹Federal State Budgetary Scientific Institution All-Russian Institute of Plant Protection; 3, h.r. Podbelskogo, St. Petersburg, 196608, Russia

²LLC Innovative Plant Protection Center; 20A, str. Pushkinskaya, St. Petersburg, 196608, Russia

³Federal State Budget Scientific Institution «North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making», 39 str. 40 Let Pobedy, Krasnodar, 350901, Russia

⁴Federal State Budgetary Institution of Science All-Russian National Research Institute of Viticulture and Winemaking “Magarach” of the Russian Academy of Sciences; 31, str. Kirova, Yalta, 298600, Russia

Abstract. This research paper presents the findings concerning the effectiveness of utilizing Pergado Zoks water dispersible granule (WDG) and Zorvec Encantia suspo-emulsion (SE) fungicides to protect grapevines against downy mildew. It shows that double or triple treatment of vines provides strong protection of the plants against the disease without adversely affecting vegetative growth of the crop. It has been proven that effective protection of vines against downy mildew through the use of Pergado Zoks WDGs and Zorvec Encantia SE has allowed for ensuring high crop-saving rate (up to 140%) as compared to control.

Resistance risk remains a pressing challenge in terms of vine protection against downy mildew. At the same time, continuous study is conducted in respect of the ecological aspects of environmental behavior of the chemical substances under consideration and their influence on the taste of products [1, 2, 3]. Hence, manufacturers of plant protection agents are constantly searching for and monitoring new active ingredients and develop pesticides which will allow for ensuring highly effective protection of vines against downy mildew and reduce the resistance risk [4].

Downy mildew is one of the most destructive vine diseases. Causal agent — *Plasmopara viticola* Berl. et de Toni. — is an obligate monophage parasitizing exclusively on grapevines. The disease develops intensively in humid climate conditions. The harm caused by downy mildew consists in reduced carbon dioxide assimilation by the infected leaves, which results in yield losses. The disease leads to sugar content reduction, causes failure of many physiological processes in plants, leaf drop, suppression of vines, and poor ripening of shoots, which results in poor establishment of crops in the next year. Yield losses due to downy mildew reach 70% in certain years in case of epiphytotic development of the disease [5, 6, 7, 8, 9].

* Corresponding author: ovk@icZR.ru

Chemical control remains the method of choice in terms of grapevine protection against downy mildew, as the most effective and less laborious approach. Fungicides are applied regularly, almost until harvest-time, which is also connected with potential retention of the infection on healthy leaves too.

Nowadays, the range of grapevine protection products comprises fungicides from different chemical classes, including combined drugs.

Since 2014, Russia has allowed utilization of the fungicides Pergado M WDG (containing 25 g/kg of mandipropamid and 245 g/kg of copper oxychloride) and Tanos WDG (containing 250 g/kg of famoxadone and 250 g/kg of cymoxanil) for protecting the crop from downy mildew.

Both the drugs are designed for protecting agricultural plants against pathogens of the Oomycetes class, which also includes the causal agent of grapevine downy mildew.

Mandipropamid, which is a component of Pergado M drug, inhibits the biosynthesis of the pathogen cell wall. Phospholipid biosynthesis disturbance is also observed. Gradual ingress of mandipropamid into plant tissues provides for anti-sporulation and curative effect. When it enters plant tissues, mandipropamid protects the lower surface of leaves too. When absorbed by the pathogen, copper oxychloride, which is a contact fungicide with preventive activity, disrupts the enzyme system of the former [10, 11].

Famoxadone is one of the components of Tanos WDG. It binds tightly to the cuticle and is retained in the waxy layer of leaves. Famoxadone mechanism of action consists in inhibition of cellular respiration and blocking of ubiquinone activity in complex III of the respiratory chain and fungal cell mitochondria. The second active ingredient — cymoxanil — has a local contact action, inhibiting sporulation of the pathogen [12, 13].

During the past decade, the use of these drugs has enabled effective protection of the crop from downy mildew, but due to the probability of resistance development by the pathogen, it is necessary to continuously improve the systems of grapevine protection against downy mildew based on the monitoring of phytosanitary condition, soil and climatic conditions, study of bioecological features of the pathogen development, and application of state-of-the-art pesticides.

Two new drugs have now for the first time entered the Russian market of plant protection agents; it is possible that these drugs will drive Pergado M and Tanos out in future. One of their formulation constituents is replaced with an entirely new active component.

For example, Pergado Zoks WDGs contain zoxamide (240 g/kg) (which belongs to the benzamide class) instead of copper oxychloride. It is a protective fungicide inhibiting the assembly of the β -tubulin protein and thus preventing normal cell division (mitosis) in plant pathogenic fungi [14].

Introduction of a new active ingredient has allowed for reducing Pergado M WDG drug application rate of 3–5 kg/ha to 0.4–0.6 kg/ha for Pergado Zoks WDG drug. Our research has shown that application rate reduction has had no adverse effect on the antifungal activity against downy mildew.

The study of the biological effectiveness was conducted in 2015–2016 in the conditions of Krasnodar region and the Republic of Crimea. The research was conducted on the following varieties: Riesling, Chardonnay, Rkatsiteli, and Aligote; they have been released for these regions and are susceptible to downy mildew. The experiments were carried out and records were made in accordance with the Guidelines for Registration Tests of Fungicides in Agriculture [15].

Both growing seasons were characterized by warm weather (the temperature was by an average of 1.3–1.8°C higher than the long-term mean annual values). 2015 was the most favorable year in terms of moisture availability in June–July, with the mean annual amount of precipitation. The same period in 2016 featured more arid conditions (19–46% of the long-term mean annual values), which adversely affected the progress of the disease.

Spraying was performed twice, during the following phases: the berries are grain-sized; the berries have reached their final size — berry ripening.

The results of the research conducted have made it clear that reduction of the application rates for the new drug has not reduced the fungicide effect against the downy mildew causal agent as compared to Pergado M WDG reference standard (Table 1).

Table 1. Effectiveness of the Use of Pergado Zoks WDG Fungicide on Grapevines Against Downy Mildew (Krasnodar region, the Republic of Crimea, 2015–2016)

| rate of application | biological effectiveness* [%] | | yield [kg/vine] | saved crop [%] |
|---|-------------------------------|-------------|-----------------|----------------|
| | leaves | bunches | | |
| Krasnodar Krai | | | | |
| 0.4 | 83.2-94.5 | 94.0 | 3.7 | 68.2 |
| 0.5 | 92.7-96.3 | 94.0 | 3.7 | 68.2 |
| 0.6 | 92.7-99.1 | 94.0 | 3.6 | 63.6 |
| 5.0 (reference standard) | 91.2-99.1 | 73.1 | 3.2 | 45.5 |
| control | 10.1–16.5** | 6.7** | 2.2 | - |
| Least Significant Difference (LSD ₀₅) = 0.7 | | | | |
| The Republic of Crimea | | | | |
| 0.4 | 72.1-76.9 | 80.8-81.2 | 5.3 | 32.5 |
| 0.5 | 74.0-81.4 | 85.5-90.5 | 5.3 | 32.5 |
| 0.6 | 77.0-81.5 | 88.1-91.8 | 5.1 | 27.5 |
| 5.0 (reference standard) | 70.9-79.6 | 83.6-88.1 | 5.4 | 35.0 |
| control | 24.4-29.9** | 15.8-22.6** | 4.0 | - |
| Least Significant Difference (LSD ₀₅) = 0.5 | | | | |

* — average value for two years

** — progress of the disease in the control, average for two years [%]

In the Krasnodar region, no significant difference was observed between the drugs in terms of their effectiveness, against the background of an average 10.1–16.5% disease progress for two years on leaves in the control. Introduction of a new active ingredient into Pergado Zoks WDG drug has allowed for retaining high efficiency (83.2–99.1%) on a par with the reference fungicide Pergado M WDG, despite a significant difference in their rates of application. The protective effect of Pergado Zoks WDG on bunches lasted longer, which manifested in higher efficiency (up to 94.0%) as compared to Pergado M WDG (73.1%).

Application of the new drug also improved the saved-crop percentage, which ranged from 63.6 to 68.2% and was on a par with the reference case (45.5%).

In the Republic of Crimea, the weather conditions contributed to more intensive progress of the disease both on leaves (24.4–29.9%) and on bunches (15.8–22.6%). Against this background, the new drug also showed high efficacy on leaves (72.1–81.5%) and on bunches (80.8–91.8%), being on a par with the reference drug performance (70.9–79.6% and 83.6–88.1% respectively). Application of the drugs provided for a significant percentage of saved crops: 27.5–32.5% (Pergado Zoks WDG) and 35.0% (Pergado M WDG).

Thus, reduction of the application rates enabled enhancement of the environmental compatibility of the plant protection agents without impairing their fungicide effect. Replacement of the copper-based inorganic compound allowed for obtaining a drug with a median lethal dose (LD₅₀) of over 5,000 mg/kg for rats. This further resulted in reduction of the toxic load from 1,600–2,696 mg/ha for Pergado M drug to 78–118 mg/ha for Pergado Zoks fungicide.

Another recently introduced low-toxicity drug is Zorvec Encantia SE fungicide; in this drug, cymoxanil, which is a component of Tanos WDG, has been replaced with a new active ingredient — oxathiapiprolin. It is a systemic fungicide which, when applied to a plant, binds to the waxy coating of the leaves and maintains the required concentration inside the plant, by penetrating the leaf tissues adjacent to the cuticle, and systemically spreads through the plant, entering the xylem vessels. The mechanism of its action consists in affecting the Oxysterol-binding protein at the molecular level; blocking of this protein first leads to arrested growth of the fungal mycelium and germ tubes of spores and then — to the death of the fungus [16].

Inclusion of this active ingredient from the new chemical class of isoxalines allows for mitigating the threat of resistance development in pathogens, in particular in the grapevine downy mildew causal agent, without reducing the effectiveness of control of this disease.

The assessment of the biological and economic effectiveness was performed in 2015–2017 in the conditions of Krasnodar region, also in accordance with the Guidelines for Registration Tests of Fungicides in Agriculture [15]. Experiments were established with Riesling, Rkatsiteli, and Chardonnay varieties.

The growing season of 2017 was characterized by rather dry weather conditions, for example June and July saw only 40% of the long-term mean annual precipitation, which adversely affected the development of downy mildew. The weather conditions of 2015 and 2016 are described above in connection with Pergado Zoks drug.

During the first two seasons, the drug under consideration was applied at the rates of 0.35, 0.5, and 0.65 l/ha, three times within the following phases: from the end of flowering until the end of bunch development. The progress of the disease in the untreated control variant averaged from 5.9 to 25.0% on leaves and from 2.1 to 27.1% on bunches (Table 2).

Table 2. Effectiveness of the Use of Zorvec Encantia SE Fungicide on Grapevines Against Downy Mildew (Krasnodar region, 2015-2017)

| rate of application [l, kg/ha] | biological effectiveness* [%] | | yield [kg/vine] | saved crop [%] |
|---|----------------------------------|-------------|-----------------|-------------------|
| | leaves | bunches | | |
| 2015 | | | | |
| 0.35 | 69.0-100 | 83.4-87.4 | 4.4 | 46.7 |
| 0.5 | 73.4-100 | 88.2-95.2 | 4.6 | 53.3 |
| 0.65 | 77.8-100 | 90.8-92.3 | 4.9 | 63.3 |
| 0.4 (reference standard) | 57.8-100 | 84.1-91.1 | 4.8 | 60.0 |
| control | 7.4-24.9** | 10.1-27.1** | 3.0 | - |
| Least Significant Difference (LSD ₀₅) = 0.7 | | | | |
| 2016 | | | | |
| 0.35 | 91.4-100 | 91.1-100 | 4.1 | 17.1 |
| 0.5 | 94.0-98.3 | 90.0-100 | 4.3 | 22.9 |
| 0.65 | 91.4-95.7 | 91.7-100 | 4.2 | 20.0 |
| 0.4 (reference standard) | 89.4-97.5 | 93.3-100 | 4.0 | 4.3 |
| control | 5.9-10.3** | 2.1-18.0** | 3.5 | - |
| Least Significant Difference (LSD ₀₅) = 0.4 | | | | |
| 2017 | | | | |
| 0.65 | 90.0-96.8 | 90.7-99.1 | 5.2 | 8.3 |
| 0.8 | 92.7-97.3 | 88.0-99.1 | 5.4 | 12.5 |
| 0.4 (reference standard) | 88.4-97.0 | 88.7-99.1 | 5.4 | 12.5 |
| control | 13.1-25.7** | 11.6-15.8** | 4.8 | - |
| Least Significant Difference (LSD ₀₅) = 0.4 | | | | |

* — average value for two years

** — progress of the disease in the control variant [%]

Against this background, the drug under consideration and the fungicide used as a reference for comparing the effectiveness have demonstrated similar high performance. Utilization of Zorvec Encantia SE fungicide on leaves allowed for obtaining a fungicide effect within the range of 69.0–100%, depending on the year and rate of application, which was on a par with the reference drug Tanos WDG in terms of effectiveness, varying between 57,8 and 100% for the latter.

On bunches, where the disease occurred somewhat later than on leaves, the effectiveness was higher and more uniform both in the variants treated with the new drug (83.4–100%) and in those treated with Tanos WDG drug (84.1–100%).

The Zorvec Encantia SE application regulations under discussion were slightly changed in 2017: the application rate of 0.8 l/ha was added. This was done based on the results obtained, in order to avoid sharp decrease in effectiveness in case of increasing disease development. Raising of the application rate allowed for obtaining more stable results in terms of reduction of the disease development in respect of the disease the effectiveness against which ranged from 91.1 to 100%.

Protection of grapevines with this drug for three years enabled obtaining of a reliable crop-saving value, which averaged 30.5% over three years.

In addition to high performance in terms of the biological and economic effectiveness, introduction of Zorvec Encantia SE fungicide allows for enhancing the environmental-friendliness of the process of protecting the crop against downy mildew. Such effect was obtained through inclusion of lower-toxicity oxathiapiprolin (whose LD₅₀ is over 5,000 mg/kg) in the drug formula. This enabled reduction of the toxic load per 1 ha in case of one-time application from 366 mg for Tanos WDG drug to 86–106 mg for the new fungicide Zorvec Encantia SE.

Based on the results obtained, it can be claimed that Zorvec Encantia SE is a fungicide equal to Tanos WDG in terms of protective capability but superior in terms of safety for the environment and human health.

Thus, as a result of the research conducted it was found that the use of Pergado Zoks WDG and Zorvec Encantia SE drugs within the regulations under consideration makes it possible to control the development of the downy mildew causal agent at an economically safe level and obtain a significant yield gain. Inclusion of these drugs in the crop protection system reduces environmental hazard of local pesticide pollution of agrocenoses.

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