

Sensitivity of *Venturia inaequalis* populations to krezoxyim methyl

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As a result of investigations carried out in the industrial orchards in 2000–2006, significant decrease of sensitivity of apple scab agent *Venturia inaequalis* populations to krezoxyim methyl (Strobi) from strobilurine group was determined. In orchard "Kletsy", Minsk district, in the first year of the application of this fungicide the amount of sensitive isolates has made 100 % and the resistance factor has not increased 24.2. After 4 times of preparation application in 2001 a proportion of sensitive isolates decreased up to 80.2 % and the population resistance factor increased 2.6 times. When Strobi application was stopped in 2002, a tendency to sensitivity decrease has remained: a proportion of sensitive isolates decreased up to 63.3 % and the resistance factor reached 88.4.

In orchard "Rassvet", Brest district, where krezoxyim methyl was started to apply since 1998, in 2005 a proportion of the resistant isolates has made 59.2 %, and the population resistance factor was 117.2. When the number of treatments decreased up to one in 2006, the situation has not changed essentially, what proves the progressive loss of *V. inaequalis* population sensitivity to the fungicide.

Key words: apple-tree, effective concentration (EC_{50}), fungicides, industrial orchards, resistance, strobilurin, *Venturia inaequalis*.

Introduction. Apple-tree is the basic fruit crop in Belarus occupying 95 % of the total area of large-and-small fruit plantations (Самусь, 2008). When there was passed to the intensive gardening in the Republic, apple scab (the agent – fungus *Venturia inaequalis* (Cooke) Winter) development in the industrial apple orchards practically annually reaches epiphytoty. Thus, crop losses on susceptible to the disease varieties reach 60 %. Under the conditions of Belarus for orchard protection against scab the limited assortment of systemic fungicides is used. To get a standard production, such preparations as Score EC from triazole group; Chorus WDG from anilino-pirimidine group and Strobi, 500 g kg⁻¹ from strobilurine group are the most often applied. Long application and repeated treatments by these fungicides promote accumulation of resistant forms in a phytopathogen population.

Fungicides from strobilurine group have appeared on the market of Belarus in the late nineties. Strobilurines are the analogues of a natural antibiotic strobilurine

A, which is produced by fungi *Strobilurus tenacellus* and *Mycena galopoda* (Bühler, Kollar, 1996). Their biochemical action – inhibition of breath Qo; therefore, to the center in cytochrome b of mitochondria the abbreviated name QoI (Qo – inhibitors) is given. To the Site-specific fungicides high risk of resistance development is characteristic. In 1998 (Ericksen, 1999) for the first time it was informed on field resistance of downy mildew of wheat to strobilurine in the North of Germany. In 2001 there were data on resistance of cucumber powdery and downy mildew agents (*Podosphaera fusca* and *Pseudoperonospora cubensis*) in greenhouses of Japan (Ishii et al., 2001). A representative of QoI-fungicides krezoxim methyl has started to be used in Europe in industrial orchards since 1996 (Kunz et al., 1998). In America the application of this fungicide in industrial orchards began in 1999, and in the experimental ones – in 1994. Since 1997 the researches on studying the mechanism of resistance occurrence and monitoring of sensitivity to strobilurine have been carried out and in 2000 a laboratory mutant of *V. inaequalis* resistant to krezoxim methyl is obtained (Olaya, Koller, 1999; Zheng, Olaya, Koller, 2000). The pathogen resistance is noted in North America (USA, Canada) and South America (Chile) (Koller et al., 2004; Sallato, Latorre, 2006; Jobin, Carisse, 2007).

In this connection the objective of our work was to carry out *V. inaequalis* resistance to strobilurin group strobi fungicide (a. i. krezoxim methyl) monitoring in industrial orchards.

Object, methods and conditions. The infected material (scab infected leaves and fruits) was selected from industrial orchards “Kletsy”, Minsk area, and “Rassvet”, Brest area, and also from private sector gardens, where there were no fungicide treatments. The monosporous isolates of *V. inaequalis* were isolated by means of a special nozzle and a cultivation method (Седов, Жданов, 1985).

The pathogen sensitivity to krezoxim methyl was estimated by the general in phytotoxicological practice method of fungus sowing on a nutrient medium (PDA), containing various krezoxim methyl concentrations (BASF Co., 500 g/kg krezoxim methyl) (Koller et al., 2004). There were 4 repetitions, control – without fungicide.

krezoxim methyl concentration from 2.5 to 200 mkg ml⁻¹ (ppm) was prepared by Strobi (BASF Co., 500g/kg krezoxim methyl) dilution in water. On 6, 12, 15, 21, 30-th day the fungus colonies were measured and the percent of growth suppression using Ebbot formula (1) and relative growth (2) were calculated.

$$T = (Dk - Df) : Dk \cdot 100; (1)$$

where T – growth suppression in comparison with the control, %; Dk – diameter of colony in the control (on agar with fungicide), mm; Df – diameter of colony in experience (on agar without fungicide), mm;

$$RG = Df : Dk \cdot 100; (2)$$

where RG – relative growth of pathogen colonies, %; Df – diameter of colony on agar with fungicide, mm; Dk – diameter of colony on agar without fungicide, mm.

EC₅₀ (Fungicide concentration, 50 % necessary for effective suppression of isolates, forms composing a population of the studied strain) was defined on a slide-rule where on axis X there were the data on fungus colonies growth suppression (%), on axis Y – concentration of studied fungicide in mkg ml⁻¹ (probit-analysis). Resistance

factor (RF) was calculated considering the relation of EC₅₀ tested isolates value to average EC₅₀ of sensitive isolates. As sensitive “wild” isolates were used, isolated from the orchard, which was not under pesticide treatments.

Sensitivity of apple scab agent conidia to krezoxym methyl was estimated by the results of their germination in a drop of fungicide solution (a hanging drop) in various concentrations. In the control ascospores and conidia were germinated in a drop of water. The results were considered in 24, 48 and 72 hours (Гольшин, 1993).

For statistic experimental results processing (В.Ф. Моисейченко, 1988) the average arithmetic and confidence intervals detection for 95 % probability level on PC using Excel 2007 was carried out.

Results. In orchard of farm “Kletsy”, Minsk area, since 2000 in the system of apple-tree protection against scab krezoxym methyl has been applied at the rate of 0.2 kg ha⁻¹. The number of treatments has made four during a season. The analysis of *V. inaequalis* sensitivity showed that at the end of vegetative period all fungus isolates were sensitive to krezoxym methyl. Thus, in 44.1 % isolates EC₅₀ reached 20 mkg ml⁻¹, in 38.2 % isolates EC₅₀ value fluctuated from 21 to 40 mkg ml⁻¹, and in 14.7 % isolates has not increased 60 mkg ml⁻¹ (Table 1). Krezoxym methyl concentration applied at orchard sprays has made 100 mkg ml⁻¹.

Table 1. Distribution of *V. inaequalis* isolates isolated from the industrial orchards by sensitivity to krezoxym methyl

1 lentelė. Iš pramoninių sodų išskirtų *V. inaequalis* izoliatų pasiskirstymas pagal jautrumą krezoksimumetiliui

Years of researches Tyrimų metai	Number of isolates Izoliatų skaičius	Number of treatments Apdorojimų skaičius	Distribution of isolates by EC ₅₀ Izoliatų pasiskirstymas pagal EC ₅₀ (%)					
			0–20 mkg ml ⁻¹	21–40 mkg ml ⁻¹	41–60 mkg ml ⁻¹	61–80 mkg ml ⁻¹	81–100 mkg ml ⁻¹	> 100 mkg ml ⁻¹
“Kletsy”, Minsk district (krezoxym methyl application since 2000)								
“Kletsy”, Minsko rajonas (krezoksimumetilas naudojamas nuo 2000 m.)								
2000	34	4	44.1	38.2	14.7	0	0	0
2001	56	4	14.3	23.2	25.0	12.5	3.6	21.4
2002	60	0	1.7	13.3	13.3	28.3	6.7	36.7
“Rassvet”, Brest district (krezoxym methyl application since 1998)								
“Rassvet”, Bresto rajonas (krezoksimumetilas naudojamas nuo 1998 m.)								
2005	49	4	0	0	10.2	16.3	14.3	59.2
2006	58	1	0	1.7	6.9	10.3	22.4	58.6

In 2001 in the same orchard also 4 krezoxym methyl treatments were done. The biological efficiency of the fungicide was high and reached 90.2 %. However, by *V. inaequalis* isolation from the infected leaves during harvesting it was established that in 21.4 % of isolates EC₅₀ value was higher than krezoxym methyl concentration, applied in orchard, what allows us to state that these isolates were resistant. Giving up krezoxym methyl treatments in 2002 has increased the quantity of such isolates 1.7 times and has made 36.7 %.

Under the conditions of Brest area (industrial orchard of the farm “Rassvet”)

in the system of orchard protection the krezoxym methyl has been applied two-four times during a season since 1998. The efficiency of a preparation was high enough – the disease development did not exceed 7 %. However, in orchard “Rassvet” in 2005 after 4 times of this fungicide application its efficiency has essentially decreased: the disease development on leaves reached 21.3 %, on fruits – 43.6 %. It was a reason for estimation the sensitivity of *V. inaequalis* populations to krezoxym methyl. It was established that a share of resistant isolates, in which EC₅₀ value exceeded 100 mkg ml⁻¹, has made 59.2 %.

In 2006 when orchard treatments were reduced up to one, the situation has not changed and the proportion of resistant isolates also remained at the same level (58.6 %).

Having generalized the data obtained during investigations, we established a considerable decrease of *V. inaequalis* sensitivity to the krezoxym methyl from strobilurine group. In orchard “Kletsky”, Minsk area, in the first year of Strobi application EC₅₀ of the pathogen population has made 26.6 mkg ml⁻¹ and the resistance factor has not increased 24.2 (Table 2).

After 4-fold application of a preparation in 2001 the average EC₅₀ value and the factor of *V. inaequalis* resistance has increased 2.6 times and has made 68.6 mkg ml⁻¹ and 62.4, accordingly.

Table 2. Sensitivity of *V. inaequalis* populations from the industrial orchards of Belarus to krezoxym methyl

2 lentelė. Pramoninių Baltarusijos sodų *V. inaequalis* populiacijų jautrumas krezoksimumetilui

Year of investigations Tyrimų metai	Number of treatments per season Apdorojimų skaičius per sezoną	Number of isolates Izoliatų skaičius	Average EC ₅₀ (mkg ml ⁻¹) EC ₅₀ vidurkis, mkg ml ⁻¹	Factor of resistance Atsparumo faktorius
“Kletsky”, Minsk district (krezoxym methyl application since 2000) “Kletsky”, Minsko rajonas (krezoksimumetilas naudojamas nuo 2000 m.)				
2000	4	34	26.6 ± 2.1	24.2
2001	4	56	68.6 ± 7.2	62.4
2002	0	60	97.2 ± 7.4	88.4
“Rassvet”, Brest district (krezoxym methyl application since 1998) “Rassvet”, Bresto rajonas (krezoksimumetilas naudojamas nuo 1998 m.)				
2005	4	49	128.9 ± 9.1	117.2
2006	1	58	121.8 ± 6.1	110.8

Note. EC₅₀ of sensitive isolates – 1.1 ± 0.3 mkg ml⁻¹

Pastaba. Jautrių izoliatų EC₅₀ – 1.1 ± 0.3 mkg ml⁻¹

When Strobi application was stopped in 2002, the tendency to sensitivity decrease remained: the average EC₅₀ value of fungus population reached 97.2 mkg ml⁻¹, and resistance factor made 88.4.

In orchard “Rassvet”, Brest area, where krezoxym methyl was applied since 1998, after four times fungicide application in 2005 the average EC₅₀ for *V. inaequalis*

reached 128.9 mkg ml⁻¹, and resistance factor – 117.2. When frequency of treatments decreased to one in 2006 the situation has not changed: EC₅₀ of the pathogen population reached 121.8 mkg ml⁻¹, and resistance factor – 110.2, what testifies the progressing loss of *V. inaequalis* population sensitivity to krezoksim methyl.

EC₅₀ of Kletsk population conidia germination in a fungicide drop has made 35 mkg ml⁻¹ (EC₅₀ of mycelium growth – 97.2 mkg ml⁻¹). EC₅₀ value of conidia germination isolated from orchard “Rassvet” in 2005 has made 118 mkg ml⁻¹, in 2006 – 94 mkg ml⁻¹ (EC₅₀ of mycelium growth – 128.9 and 121.8 mkg ml⁻¹, accordingly). Thus, the obtained results confirmed the presence of forms resistant to krezoxym methyl in the analyzed populations.

Since strobilurine action is directed basically on pathogen conidia germination suppression, we carried out trials studying conidia sensitivity to krezoxym methyl.

At krezoxym methyl concentration 500 mkg ml⁻¹, that is, 5 times exceeding the used in a orchard, 1.5 % of conidia isolated from the infected apple-tree samples from orchard “Kletsky”, Minsk district, has germinated and 5.5–7.2 % from orchard “Rassvet”, Brest district (Fig.).

At 100 mkg/ml concentration – 32.8 % of *V. inaequalis* conidia from orchard “Kletsky” and 47.9–54.3 % of the pathogen conidia from orchard “Rassvet” has germinated.

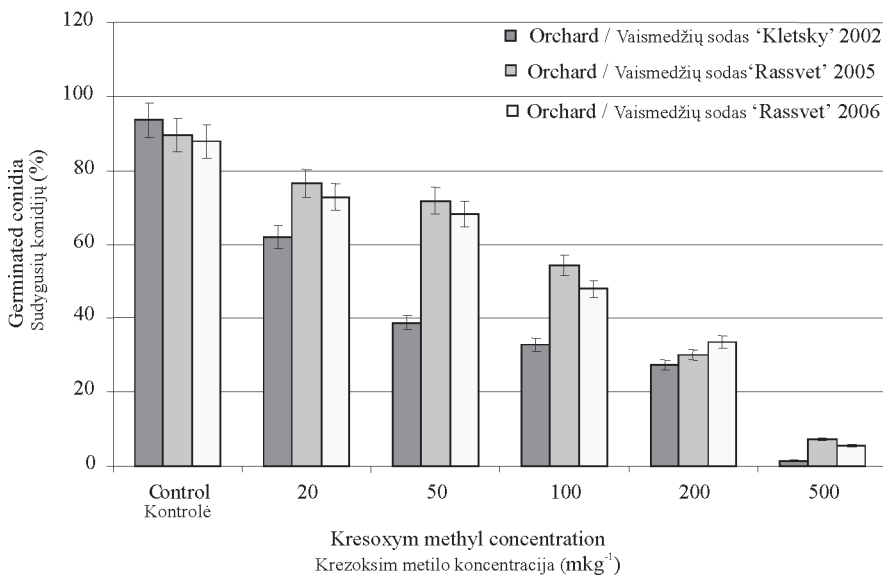


Fig. Germination of *V. inaequalis* conidia in different krezoxym methyl concentrations (laboratory trial)

Pav. *V. inaequalis* konidijų sudygimas skirtingose krezoksime metilo koncentracijose (laboratorinis bandymas)

Discussion. On getting the messages on apple scab agent resistance to QoI-fungicides, an intensive monitoring took place in Europe. The diverse levels of resistance were found in all countries of the European Union from zero and up to the highest level even within one orchard (Broniarek-Niemiec, Bielenin, Dyki, 2002).

During our researches it was also determined, that in the first year of application krezoxym-methyl efficiency against apple scab was rather high and all pathogen isolates were susceptible to the given fungicide. However, the longer this preparation is used in orchards (even at the allowed 4-times spraying), the lower susceptibility of the fungi *V. inaequalis* to the fungus.

The researches of W. Koller et al.(2004) showed that *V. inaequalis* develops 2-stage resistance to strobilurine: quantitative and qualitative (Koller et al., 2004). The quantitative answer of the pathogen population is stipulated by quantity of the done treatments in the orchards by these fungicides, and qualitative – by mutation presence in the site G143A of mitochondrial *V. inaequalis* in cytochrome b, then a fungus get an alternative breath.

Our obtained results proved the existence of a quantitative resistance (loss of sensitivity with the increase of treatments number) in the fungus *V. inaequalis*. However, it is necessary to continue the problem study and determine if resistance is qualitative to krezoxym methyl of apple scab causal agent.

On the base of carried out monitoring it is not impossible to predict the development of apple scab causal agent resistance to krezoxym methyl, but it is only possible to receive the information about the geographical distribution of the stable strains depending on the number of carried out treatments by this preparation, population resistance level changes in years, using it for substantiation the strategy and tactics of different mechanism of action fungicides application.

Conclusions. A considerable decrease of sensitivity in apple scab agent *V. inaequalis* populations to krezoxym methyl (fungicide Strobi) from strobilurine group is revealed.

The average EC_{50} value of isolates isolated from the orchard where krezoxym methyl was applied 4 times within 2 years has made from 26.6 to 97.2 mkg ml⁻¹. In 21.4–36.7 % of isolates EC_{50} increased the preparation concentration used in orchards (100 mkg ml⁻¹).

Germination of 1.5 % conidia is noted at fungicide concentration 5 times exceeding the one applied in production.

In orchards where krezoxym methyl was applied in the same frequency rate during 7 years, EC_{50} value increased to 121.8–128.9 mkg ml⁻¹, number of isolates with $EC_5 > 100$ mkg ml⁻¹ have made 58.6–59.2 %, and at concentration EC_{50} exceeding the one applied in production 5 times the amount of germinated conidia has made 5.5–7.2 %, what testifies to the progressing loss of sensitivity of *V. inaequalis* population to krezoxym methyl.

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***Venturia inaequalis* populiacijų jautrumas krezoksym metilui**

V. Kamardzina

Santrauka

2000–2006 metais pramoniniuose soduose atliktų tyrimų metu buvo nustatytas reikšmingas obelių rauplių sukėlėjo *Venturia inaequalis* populiacijų jautrumo krezoksym metilui (Strobi) iš strobilurino grupės sumažėjimas. Sode “Kletsky” (Minsko rajonas) pirmaisiais šio fungicido naudojimo metais jautrių izoliatų kiekis sudarė 100 %, o atsparumo faktorius neviršijo 24,2. 2001 metais panaudojus preparatą 4 kartus, jautrių izoliatų proporcija sumažėjo iki 80,2 %, o populiacijos atsparumo faktorius padidėjo 2,6 karto. Kai 2002 metais Strobi naudojimas buvo nutrauktas, jautrumo mažėjimo tendencija išliko: jautrių izoliatų proporcija sumažėjo iki 63,3 %, o populiacijos atsparumo faktorius pasiekė 88,4.

Sode “Rassvet” (Bresto rajonas), kuriame krezoksym metilas buvo pradėtas naudoti nuo 1998 metų, 2005 metais atsparių izoliatų proporcija sudarė 59,2 %, o populiacijos atsparumo faktorius buvo 117,2. Kai 2006 metais apdorojimų skaičius sumažėjo iki vieno, situacija iš esmės nepasikeitė, o tai rodo mažėjantį *V. inaequalis* populiacijos jautrumą šiam fungicidui.

Reikšminiai žodžiai: atsparumas, fungicidai, obelys, pramoniniai sodai, strobilurinas, veiksminga koncentracija (VK_{50}), *Venturia inaequalis*.