

Efficacy of Strobilurin-related and Multi-site Fungicide Mixtures Against Apple Scab

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SUMMARY

The efficacy of several fungicide mixtures in controlling *Venturia inaequalis* in apple was evaluated in field trials. The efficacies of Flint Plus (trifloxystrobin + captan) and Tercel (pyraclostrobin + dithianon) in comparison with standard fungicides Zato 50-WG (trifloxystrobin) and Stroby + Delan (kresoxim-methyl + dithianon) were tested in the localities Mihajlovac, Radmilovac and Landol in 2004 and 2005. Both tested fungicides exhibited high efficacy in controlling apple scab. There were significant differences in the efficacies of Flint Plus (91.3-98.5%) and Zato 50-WG (68.2% and 78.4%); and Tercel (88.7-93.5%) and Stroby + Delan (77.9% and 82.1%). Our experiments showed that the investigated fungicide mixtures are highly effective against *V. inaequalis*, even under high disease pressure.

Keywords: Apple scab; Fungicide mixtures; Trifloxystrobin + captan; Pyraclostrobin + dithianon; Efficacy

INTRODUCTION

Scab caused by *Venturia inaequalis* (Cooke) G. Winter is recognized as the most important disease of apples in economic terms. Fungicides remain the primary tool for managing this disease and, due to its polycyclic nature, repeated applications are required over the course of a single season. This intensive use of fungicides has been responsible for a more or less rapid development of resistance to several classes of specific fungicides, such as benzimidazoles and sterol demethylation inhibitors (DMIs) (Köller et al., 1997; Köller and Wilcox, 2001).

In 1999, the class of strobilurin-related fungicides, such as kresoxim-methyl and trifloxystrobin, was intro-

duced as new tools for managing scab and other apple diseases. Strobilurin analogues are derived from the natural antifungal antibiotic strobilurin A isolated from the fungus *Strobilurus tenacellus*. They belong to different chemical groups with a common biochemical mode of action on the fungus. They block electron transfer at the site of quinol oxidation (the Qo site) in the cytochrome bc1 enzyme complex in the respiratory chain of fungal mitochondria (Köller et al., 2004). These fungicides are now more appropriately referred to as QoI (Quinone outside Inhibitors) fungicides. Representatives of this new class are kresoxim-methyl and trifloxystrobin. They belong to the chemical group oximino-acetamides. In contrast to curative fungicides (captan, mancozeb, meti-

ram, propineb, etc.), strobilurin analogues are highly effective on spore germination at very low doses and are excellent preventive fungicides. Some other chemical groups with the same mode of action are also in use against apple scab: methoxy-carbamates (pyraclostrobin) and methoxyacrylates (picoxystrobin) (Anonymous, 2007a). The early stages of fungal development (spore germination, germ tube elongation and appressorium formation) are also strongly inhibited by these compounds. However, a shift in sensitivity of *V. inaequalis* to strobilurins in Germany (Kunz et al., 1998) and USA (Köller et al., 2004), after a few years of use, has stressed the importance of introducing anti-resistance strategies. In anticipation of resistance development of *V. inaequalis* to QoI fungicides, their mixtures with multi-site fungicides such as captan and dithianon were suggested as an anti-resistance strategy (Anonymous, 2007b).

Therefore, the objectives of this study were to evaluate the efficacy of new mixtures of fungicides: trifloxystrobin + captan, and pyraclostrobin + dithianon against apple scab in commercial orchards in Serbia in 2004 and 2005.

MATERIAL AND METHODS

Location

Four tests were conducted in commercial apple orchards (cv. Idared and Gloster) in Mihajlovac (Smederevo), Radmilovac (Vinča) and Landol (Smederevo) in 2004 and 2005. The tests were arranged in a randomized complete block design with four replications, according to EPPO methods (EPPO, 1997a). Individual treatment-blocks consisted of five trees.

Fungicides

Trifloxystrobin (500 g/kg WG, Zato 50°, Bayer CropScience) and the fungicide mixtures trifloxystrobin + captan (40 g/kg + 600 g/kg WG, Flint Plus® Bayer CropScience), pyraclostrobin + dithianon (40 g/kg + 120 g/kg WG, Tercel®, Basf), and kresoxim-methyl (50% WG, Strobys®, Basf) + dithianon (66% WG, Delan®, Basf) were tested in this study.

Applications

Treatments were carried out using a motorized knapsack sprayer (Solo Port 423, Germany) by thoroughly wetting the trees (water volume: 1000 liter/ha). Initiation of application was generally adjusted to standard local practice, starting from BBCH (Biologische Bundesanstalt, Bundesdienstamt and Chemical Industry) 54-55 (first leaves) until BBCH 69-71 (fruit setting). Application details are listed in Table 1.

Evaluation

In each assessment, 200 leaves of long shoots and rosettes per plot were evaluated. The percentage of disease development of the leaves was rated on a scale developed by Croxall et al. (1953): 0 (no disease), 1% (one to four small spots on about a quarter of the leaves), 5% (almost every leaf infected with scab areas covering approximately 25% of the leaf surface on about one-quarter of the leaves), 10% (every leaf infected with scab areas covering approximately 25% of the leaf surface on half the leaves), 25% (every leaf infected with scab areas covering 50% of the leaf surface on half the leaves), and 50% (all leaves infected, with scab areas covering 50% of the leaf surface on half the leaves).

Table 1. Application details for evaluation of fungicides against apple scab
Tabela 1. Uslovi primene za ocenu fungicida protiv čađave pegavosti jabuke

Year Godina	Locality Lokalitet	Date of evaluation Vreme ocene	Application interval (days) Intervali primene (dani)	Number of applications Broj tretmana	Date of application Vreme primene
2004 ^{a)}	Mihajlovac	May 11	6-8	5	Apr. 2, 8, 16, 22, 28
2004	Radmilovac	May 19	6-8	5	Apr. 7, 15, 23, 30; May 6
2005 ^{b)}	Landol	May 25	7-9	4	Apr. 22, 30 May 9, 16
2005	Radmilovac	May 31	7-10	4	Apr. 22, 30 May 10, 17, 24

^{a)} = Begin of flowering: April 16/Početak cvetanja: 16. april

^{b)} = Begin of flowering: April 23/Početak cvetanja: 23. april

Table 2. *V. inaequalis* – Disease intensity on apple leaves and fungicide efficacy (locality: Mihajlovac and Radmilovac, 2004)**Tabela 2.** *V. inaequalis* – Intenzitet zaraze na lišću jabuke i efikasnost preparata (lokalitet: Mihajlovac i Radmilovac, 2004. godine)

Treatment Varijanta	Rate Količina (kg/ha)	Mihajlovac		Radmilovac	
		Disease severity (%) Intenzitet oboljenja (%)	Efficacy (%) Efikasnost (%)	Disease severity (%) Intenzitet oboljenja (%)	Efficacy (%) Efikasnost (%)
Flint Plus	1.25	7.2 a	91.3	5.5 a	92.9
Flint Plus	1.5	3.4 a	95.9	2.9 a	96.2
Flint Plus	1.87	1.3 a	98.5	2.2 a	97.2
Zato 50-WG	0.15	17.9 b	78.4	24.7 b	68.2
Untreated Kontrola	-	82.5 c	-	77.5 c	-
LSD		7.9		7.4	

Mean values in columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's test
Srednje vrednosti u kolonama označene sa različitim slovima se statistički značajno razlikuju u skladu sa Dankanovim testom

The efficacy of the compounds tested for apple scab control was expressed in terms of disease severity using the Townsend-Heuberger's formula:

$$ID = \frac{\sum(nv)100}{NV}$$

where: n = degree of infection according to the 6-grade scale, v = number of leaves per category, V = total number of leaves screened, N = highest degree of infection. The efficacy was evaluated using Abbott's formula (Abbott, 1925). Data were analysed separately for each trial using ANOVA and the means were separated by Duncan's multiple range test (EPPO, 1997b, 1997c).

RESULTS

Climatic conditions in 2004 and 2005 were quite favourable for disease development, with frequent wet spells, in late March and early April. Under such climatic conditions, an epidemic began with initial ascospore infections, detected as a diffused outbreak of symptoms on the leaves at the end of April. This situation led to a general spread of the disease, with a very high degree of infection of leaves. Under such an exceptionally high infection, the tested fungicide mixtures provided very good protection.

Table 2 summarizes the data of disease intensity on apple leaves (Figure 1 and 2) and fungicide efficacy in the localities Mihajlovac and Radmilovac in 2004. The results show a significant difference in the efficacies of the tested fungicides Flint Plus (91.3-98.5%) and Zato 50-WG (68.2% and 78.4%). There was no significant

difference in the efficacies of Flin Plus applied in different rates.

Table 3 summarizes the data on disease intensity on apple leaves and fungicide efficacy in the localities Landol and Radmilovac in 2005. The results show a significant difference between the efficacies of the tested fungicide Tercel (88.7-93.5%) and fungicide mixture Stroby + Delan (77.9% and 82.1%). There was no significant difference between the efficacies of Tercel applied at different rates.

**Figure 1.** Control – untreated plants (locality: Radmilovac, 2004)**Slika 1.** Kontrola – netretirane biljke (lokalitet: Radmilovac, 2004)



Figure 2. Treatment – trifloxystrobin + captan (1.87 kg/ha) (locality: Radmilovac, 2004)

Slika 2. Varijanta – trifloksistrobin + kaptan (1.87 kg/ha) (lokalitet: Radmilovac, 2004)

DISCUSSION

This study shows that the fungicide mixtures tested are highly effective against *V. inaequalis*, even under high disease pressure, confirming and extending the data obtained in previous trials conducted on apple in Italy (Bologna) (Brunelli et al., 2003). The new products showed remarkable activity against the disease, often exceeding the previous standards (Zato 50-WG and Strobry) and ensuring longer intervals of protection during periods of high epidemic risk (6-10 days).

Some previous trials, carried out in several locations in Serbia in 1998/99 and 2002, also showed that stro-

bilurin fungicides (trifloxystrobin and kresoxim-methyl) were most effective in apple scab control (Balaž and Knežević, 2003). However, our results indicated a decreasing efficacy of trifloxystrobin when applied alone in the locality Radmilovac. Without further solid evidence, however, we can only assume that the time of application, climatic conditions and equipment for application were the factors causing it.

In 2000, a decreased efficacy of another member of the strobilurin group of fungicides, kresoxim-methyl, against apple scab, was reported in the apple growing region Altes Land in Northern Germany. Studies of the resistance mechanism revealed that a partial loss of efficacy of kresoxim-methyl had been caused by an external esterase that was able to metabolise the fungicide (Jabs et al., 2001). Systematic studies showed that the esterase was specifically effective in cleaving the esterbond in the toxophore of kresoxim-methyl but was much less effective in the case of other strobilurins with an esterbond such as trifloxystrobin. This finding has been confirmed by field efficacy tests. In plantations where kresoxim-methyl showed some loss of efficacy due to metabolism, trifloxystrobin was always able to achieve perfect control. So, this resistance mechanism seems to be specific for kresoxim-methyl under practical conditions and a positive cross-resistance between kresoxim-methyl and trifloxystrobin and other strobilurins does not exist (Kuck and Mehl, 2003).

Resistance management of all QoI fungicides is coordinated by the FRAC QoI Working Group. The group publishes regular reports on worldwide sensitivity monitoring and recommends resistance management tools to be used (Anonymous, 2007b). The main tools for lowering the resistance risk of QoI fungicides are: a rigid limitation of the number of treatments per

Table 3. *V. inaequalis* – Disease intensity on apple leaves and fungicide efficacy (locality: Landol and Radmilovac, 2005)

Tabela 3. *V. inaequalis* – Intenzitet zaraze na lišću jabuke i efikasnost preparata (lokalitet: Landol i Radmilovac, 2005. godine)

Treatment Varijanta	Rate Količina (kg/ha)	Landol		Radmilovac	
		Disease severity (%) Intenzitet oboljenja (%)	Efficacy (%) Efikasnost (%)	Disease severity (%) Intenzitet oboljenja (%)	Efficacy (%) Efikasnost (%)
Tercel	2.0	2.8 a	88.7	2.4 a	89.7
Tercel	2.5	1.9 a	92.3	1.5 a	93.5
Strobry + Delan	0.2 + 0.5	5.4 b	77.9	4.1 b	82.1
Untreated Kontrola	-	24.6 c	-	23.0 c	-
LSD		2.4		1.6	

Mean values in columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's test

Srednje vrednosti u kolonama označene sa različitim slovima se statistički značajno razlikuju u skladu sa Dankanovim testom

season; alternating programmes of non-cross-resistant partner fungicides in crops with a higher number of fungicide treatments; the use of mixtures of multi-site fungicides or non-cross-resistant specific fungicides in crops with a limited number of fungicide treatments. We should add that all partner fungicides have to be used at a sound rate, which is sufficient to guarantee a solid pathogen control when used alone at the same rate as in the QoI resistance management programme. Information on QoI resistance development and adequate resistance management recommendations are being updated annually by the FRAC QoI Working Group on their internet site (Kuck and Mehl, 2003).

REFERENCES

- Abbott, W.S.:** A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 1925.
- Anonymous:** Frac code list 2: Fungicides sorted by modes of action, <http://www.GCPF.org/FRAC/FRAC.html>, Accession date: July 15, 2007a.
- Anonymous:** Recommendations of the FRAC QoI Working Group for 2003. www.frac.info, Accession date: July 25, 2007b.
- Balaž, J. and Knežević, T.:** Efikasnost novijih fungicida u suzbijanju čađave krastavosti i pepelnice jabuke. *Pesticidi*, 18: 175-185, 2003.
- Brunelli, A., Gianti, P., Flori, P. and Berardi, R.:** Experimental trials on the activity of new fungicides against apple scab. *Pflanzenschutz-Nachrichten Bayer*, 56: 259-280, 2003.
- Croxall, H.E., Gwynne, D.C. and Jenkins, J.E.:** The rapid assessment of apple scab on fruit. *Plant. Pathol.*, 2: 89-92, 1953.
- EPPO:** Guidelines for the efficacy evaluation of plant protection products: *Venturia inaequalis* and *V. pirina* – PP 1/5(3). In OEPP/EPPO Standards: Guidelines for the efficacy evaluation of plant protection products, 2, EPPO, Paris, 1997a, pp. 86-90.
- EPPO:** Guidelines for the efficacy evaluation of plant protection products: Design and analysis of efficacy evaluation trials – PP 1/152(2). In EPPO Standards: Guidelines for the efficacy evaluation of plant protection products, 1, EPPO, Paris, 1997b, pp. 37-51.
- EPPO:** Guidelines for the Efficacy Evaluation of Plant Protection Products: Conduct and Reporting of Efficacy Evaluation Trials – PP 1/181(2). In: EPPO Standards: Guidelines for the efficacy evaluation of plant protection products, 2, EPPO, Paris, 1997c, pp. 52-58.
- Jabs, T., Cronshaw, K. and Freund, A.:** New strobilurin resistance mechanism in apple scab (*Venturia inaequalis*). *Phytopathology*, 31: 15-16, 2001.
- Köller, W., Wilcox, W.F., Barnard, A.L. and Braun, P.G.:** Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. *Phytopathology*, 87: 184-190, 1997.
- Köller, W. and Wilcox, W.F.:** Evidence for the predisposition of fungicide-resistant phenotypes of *Venturia inaequalis* to a preferential selection for resistance to other fungicides. *Phytopathology*, 91: 776-781, 2001.
- Köller, W., Parker, D.M., Turechek, W.W., Avila-Adame, C. and Cronshaw, K.:** A two-phase resistance response of *Venturia inaequalis* populations to the QoI fungicides kresoxim-methyl and trifloxystrobin. *Plant Dis.*, 88: 537-544, 2004.
- Kuck, K.H. and Mehl, A.:** Trifloxystrobin: Resistance risk and resistance management. *Pflanzenschutz-Nachrichten Bayer*, 56: 313-325, 2003.
- Kunz, S., Lutz, B., Deising, H. and Mendgen, K.:** Assessment of sensitivities to anilinopyrimidine- and strobilurin- fungicides in populations of the apple scab fungus *Venturia inaequalis*. *J. Phytopathol.*, 146: 231-238, 1998.

Efikasnost kombinacija nespecifičnih i fungicida iz grupe strobilurina u suzbijanju čađave pegavosti jabuke

REZIME

Ispitivana je efikasnost nekoliko kombinacija fungicida u suzbijanju *Venturia inaequalis* na jabuci u poljskim uslovima. U 2005-oj i 2006-oj godini ispitivana je efikasnost Flint Plus (trifloksistrobin + kaptan) i Tercel (piraklostrobin + ditianon) u odnosu na standardne fungicide Zato 50-WG (trifloksistrobin) i Strobry + Delan (kresoksim-methyl + ditianon) na lokalitetima Mihajlovac, Radmilovac i Landol. Oba ispitivana fungicida ispoljila su visoku efikasnost u suzbijanju čađave pagavosti jabuke. Između efikasnosti Flint Plus (91.3-98.5%) i Zato 50-WG (68.2% i 78.4%), i Tercel (88.7-93.5%) i kombinacije fungicida Strobry + Delan (77.9% i 82.1%) zabeležena je statistički značajna razlika. Naši ogledi su pokazali da su ispitivane kombinacije fungicida visoko efikasne protiv *V. inaequalis* čak i u uslovima jake zaraze.

Ključne reči: Čađava pegavost; kombinacije fungicida; trifloksistrobin + kaptan; piraklostrobin + ditioanon; efikasnost