

COMPARING OF SOME FUNGICIDE TREATMENT PROGRAMS FOR MANAGING THE INFECTIONS FROM *VENTURIA INAEQUALIS* IN STARKING APPLES

Edmond Rexhepi^{1*}, Harallamb Paçe¹, Hekuran Vrapit¹, Arbenita Hasani²

¹Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirana, Albania

²Department of Food Technology, Faculty of Agriculture and Veterinary, University of Prishtina, Prishtina, Kosovo

Abstract. Apple scab caused by the pathogen of fungus *Venturia inaequalis* that is spread from airborne spores is a continuous threat to the apple production in Kosovo country regions. The disease causes scabby dark marks on the leaf and fruit making the fruits non marketable. In order to be controlled, this disease needs to be treated with plant protection products (PPP) in many applications per cultivation season. The aim of this study was to identify and verify the best treatment program for apple scab disease control that provides the lower disease index on the treated trees. For this purpose, in this research eight treatment programs were used consisting with different fungicides and different treatment periods. The experimental design was set up in two factorial randomized block with four replications, with main effect factor the treatment periods (A) in four levels and the second effect factor (B) the treatment programs in eight levels. By analyzing the disease index for each treatment program, it resulted that the treatments applied to the trees based on the warnings provided by one decision support system (DSS) had the lowest disease index (DI%) and needed the lowest number of spraying applications for the scab control.

Keywords: infection, index, disease, program, scab, treatment.

Corresponding Author: Edmond Rexhepi, Department of Plant Protection, Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirana, Albania, e-mail: rexhepiedmond@yahoo.com

Received: 24 September 2018; **Accepted:** 29 October 2018; **Published:** 21 December 2018.

1. Introduction

Apple scab, caused by the fungus *Venturia inaequalis* (Cke.) Wint., is a yearly threat to apple production (Agrios, 2005). The symptoms are generally most noticeable and serious on leaves and fruits. The first lesions seen in the spring are usually on the underside of expanding leaves. Once the leaves open, the upper surfaces also become vulnerable to infection. A lesion first appears as an area which is a lighter shade of green than the surrounding leaf. The lesion is usually circular and as it increases in size it becomes olive-colored and velvety due to production of asexual spores (conidia). Lesions that are formed on young leaves may be quite large, some more than 1 cm in diameter. Lesions that are formed on expanded leaves are usually smaller because older leaves are more resistant to infection. Affected tissues eventually may become distorted and puckered, and the leaf lesions often become cracked and torn. Lesions on the leaves and fruit are generally blistered and "scabby" in appearance, with a distinct margin (Vaillancourt & Hartman, 2010). The symptoms on leaves from experimental orchard are shown in Figure 1. Once present on the fruit itself, the lesions are round, velvety and olive green turning darker, scabby and often cracked. Later in the spring, these primary

infections produce secondary spores which infect other leaves and fruits. These secondary infections may continue throughout the growing season during wet periods. (Jamar, 2011).



Figure 1. Scabby marks on Starking leaves and fruits from the experimental orchard

In the region of Gjilan in Kosovo, one of the major fruit that is cultivated in commercial orchards is the apple fruit. It almost represents 60% of the total number of fruit cultivars. The Starking is cultivar is dominative then followed by Golden Delicious, Gala, Fuji, Granny Smith, etc. Taking in to the consideration that Starking cultivar is being cultivated mostly in the country, the apple scab disease presents a serious risk for local growers. The apple cultivation is increasing every year including the other apple cultivars that are being planted but in small numbers. According to Rexhepi and Paçe (2017), one of the major challenges for apple cultivation farmers in this region and overall in Kosovo remains the managing of the *V. inaequalis* infections, especially in rainy seasons which causes the reduction of apple fruit production and market value. If the disease is not controlled, over 80% of fruits of susceptible cultivars can be damaged. Depending on the risk of the disease, 10 to 15 or even more fungicidal applications are usually needed for efficient control (Meszka, 2015). The number of treatments depends on cultivar susceptibility, the amount of source infection and weather conditions, mainly air temperature, leaf wetness, relative humidity and rainfall (MacHardy & Gadoury, 1996).

The overall goal of this study was to develop one treatment program with available plant protection products from market and finding the optimal time frame to realize the fungicide application for managing the *V. inaequalis* primary and secondary infections. This research had two main objectives. The first objective was to establish action thresholds for initiation of the treatments. The second objective was to combine several fungicides that would fit in the established treatment period.

Since in this cultivation zone, up to now there were not conducted any similar research for apple or pear scab disease, this research is meant to provide helpful information for the apple growers and extension services.

2. Materials and methods

The research field work was carried out during the cultivation season of 2017 in one experimental apple orchard in cultivation with apple Starking cultivar in Zhegra (N 42.378998°, E 21.485185°), region of Gjilan, Kosovo. This orchard had developed apple scab disease in previous seasons. The design of the experiment is two factorial

randomized block with four replications and is formatted with Factor A for the treatment period in four levels: 1. RIM-pro period (relative infection measure-program) is developed by Bio-Fruit-Advies in Netherlands which is a decision support system (DSS) as a cloud platform that provides warnings for *Venturia* primary and secondary infection periods to the farmers and researchers all over the Europe that are connected online. 2. The phenological phases of cultivar and for this research are used only six phenological phases from BBCH Scale: 10, 67-69, 71, 72, 74 and 85-87 (Meier, 2001). 3. Local farmer's traditional treatment period. 4. Control-untreated apple trees. The second effect factor (B) is treatment programs in eight levels with combination of several plant protection products as shown in table 1. The volume of products were prepared and mixed as per manufacturer's recommendation on the product label.

Table 1. Treatment programs with fungicide combination

Program	Fungicide	Active substance	Activity	Producer
1	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Syllit 400SC	Dodine 400g/l	Contact	Agriphar
2	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Captan 80WG	Captan 800g/kg	Contact	Arysta Lifescience
3	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Mankosav 80WP	Mancozeb 800g/kg	Contact	Agrosava
4	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Folicur 250EW	Tebuconazole 250g/l	Systemic	Bayer
	Captan 80WG	Captan 800g/kg	Contact	Arysta Lifescience
5	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Antracol 70WP	Propineb 700g/kg	Contact	Bayer
	Score 250EC	Difconazole 250g/l	Systemic	Syngenta
6	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Zato 50WG	Trifloxystrobin 500g/kg	Systemic	Bayer
	Daconil 720SC	Chlorothalonil 720ml/l	Contact	Syngenta
7	Champion 50WG	Copper hydroxide 500g/kg	Contact	Nufarm
	Chorus 50WG	Cyprodinil 500g/kg	Systemic	Syngenta
	Daneel 700WG	Dithianon 700g/kg	Systemic	BASF
8	Control	no fungicides	-	-

On the 22 July 2017, there were randomly picked up by 25 leaves from each apple tree (by all sides of the tree) from the randomized block in order to determine the scab severity. On the 23 September 2017 which corresponds with the fruit harvest time, by 10 fruits per tree were randomly picked up from the same trees that were previously picked the leaves for analysis. In the laboratory 2400 leaves and 960 fruits were analyzed for the disease infection level which were taken from total of 98 apple trees from the experimental plot.

For every treatment program, the category of classification of the disease severity was determined based on the organ (leaf and fruit) surface area infected by fungus *Venturia inaequalis*. Disease severity is a measure of the amount of disease per sampling unit (Nutter *et al.*, 2006). The Croxall *et al.* (1952) reported a standard diagram method for rating the scab severity. The Lateur and Blazek (2002) reported standard area diagram (SAD) from 1 to 9 scab intensity scale. This scale is modified

from 9 to 6 SAD categories and is presented in percentage from 0% to 75% of the fruit surface infected area as shown in table 2 (Hasani, 2005).

Table 2. SAD categories and levels of classification for scab infection assessment

Category	Intensity level	Infection level (severity)
0	Nothing noticed	0% of leaf or fruit surface infected
1	Light intensity	0.1 - 10% of leaf or fruit surface infected
2	Medium intensity	10.1 - 25% of leaf or fruit surface infected
3	Strong intensity	25.1 - 50% of leaf or fruit surface infected
4	Very strong intensity	50.1 - 75% of leaf or fruit surface infected
5	Destructive intensity	> 75% of leaf or fruit surface infected

Through pondered average of McKinney's formula (McKinney, 1925) which is later modified by B.M Cooke (Cooke et al., 2006) is calculated the disease index.

$$I = \frac{\sum (ni \times ki)}{N \times K} \times 100$$

I = disease index; ni = number of leaves or fruits in respective category; ki = number of each category; N = total number of leaves/fruits analyzed; K = total number of categories.

Table 1. Treatment periods with dates of application

Treatment Periods	Dates during 2017
1 RIM pro (DSS)	04.04; 22.04; 15.05; 31.05; 16.06
2 Six Phenological phases	20.03; 15.05; 01.06; 16.06; 05.07; 30.08
3 Local farmer	12.03; 25.03; 15.04; 08.05; 28.05; 15.06; 05.07; 15.08

In order to make sure the infection assessment based on SAD is accurate, it was used the Leaf Doctor software (Pethybridge *et al.*, 2015) to compare some of the diseased leaves and fruits.

The weather conditions in the orchard are measured and collected by individual weather station model i-Metos 2 produced and configured by Pessl Instruments from Austria and monitored by Field-Climate which is a cloud platform from the same company. The weather station was set up in the middle of the orchard, 2m above the ground. The ascospores trapping was performed with one simple spore-trap partially made from the glass and wood material (Osprey *et al.*, 1992) and was positioned in the orchard center. Later, the glass slides were observed in the microscope to identify the captured ascospores in the early spring. The other verification was with visual inspection aiming to find and evidence of the first conidia spots or lesions on the infected leaves and the apple trees fruits.

The statistical data analysis for this research respectively the averages, variance and standard deviation are calculated with statistical program Assistat 7.7 and the comparison of disease index averages was completed using the Tukey-Kramer HSD test for two levels of probability $P = 0.05$ and $P = 0.01$ with JMP® 14.0 from SAS 2009.

3. Results and discussion

The weather conditions such as temperature, rainfall and relative humidity during 2017 and especially for the months that apple scab primary and secondary infections are mostly developed are measured on the orchard and are presented in below table. The seasonal weather data's for apple cultivation presented in table 4 show that the natural conditions for development of primary infection from the ascospores and secondary infection from the conidia's of fungus *Venturia inaequalis* in the apple orchard were optimal and we had the development of the apple scab infection. This fact was verified also by monitoring the discharged ascospores captured by the spore trap.

Table 2. Weather data by 10-day period per month measured on the experimental orchard

2017	T °C Max	T °C Avg.	T °C Min	RH% Max	RH% Avg.	RH% Min	Rain (mm)
February I	16.9	3.76	-5.5	95.72	81.95	34.2	17.2
February II	14.5	2.18	-6.8	89.97	72.5	23.3	15.2
February III	19.9	6.89	-6.2	97.41	72.6	21.9	6.4
March I	19.0	8.1	-2.8	90.16	64.57	37.74	10.0
March II	24.1	11.1	-1.9	90.66	66.67	41.22	3.6
March III	26.9	12.25	-2.4	92.34	57.76	23.58	7.6
April I	23.7	12.15	0.6	91.92	60.22	31.27	39.0
April II	26.1	12.25	-1.6	95.43	64.54	31.23	25.4
April III	30.4	13.45	-3.5	97.29	66.29	32.46	2.4
May I	29.5	17.15	4.8	99.5	76.65	37.37	29.8
May II	29.5	15.8	2.1	99.19	68.68	31.38	17.8
May III	32.8	19.15	5.5	99.2	78.96	42.73	54.4
June I	33.7	20.25	6.8	99.3	77.39	35.37	59.4
June II	33.6	21.5	9.4	99.52	76.3	40.72	17.8
June III	39.2	25.05	10.9	98.07	64.29	25.2	5.2
July I	42.1	25.05	8.0	97.64	63.11	27.87	8.8
July II	39.9	24.75	9.6	92.9	58.63	28.59	10.0
July III	40.2	25.5	10.8	95.0	56.37	21.91	0.4
August I	42.7	26.08	11.1	89.82	48.04	14.39	0.0
August II	40.9	25.7	10.5	97.95	63.86	28.73	44.4
August III	37.7	21.6	5.5	97.59	60.44	21.66	10.4

The leaves assessment result for the Apple scab disease for the susceptible Starking cultivar are presented on table 5. The disease index DI% varies from 12.0%, 13.05% and 15.85%. They are all listed in class E as per Tukey-Kramer HSD test for the first treatment program with the same fungicides such as Copper hydroxide and Dodine but with different treatment period (fungicide application). In comparison to the second treatment program with other fungicide combination which is in class D as per Tukey-Kramer HSD test compared to other treatment programs that are in other classes with higher disease index values. It resulted that such programs as 5, 6 and 7 have no statistical difference between each other. Finally, all treatment programs were compared with the control variant.

Table 3. Disease Index (DI %) on leaves of apple cv. Starking analyzed on July 2017

Treatment Programs		Treatment Periods		
		RIMpro	Phenological phases	Local farmer
		Avg. DI%	Avg. DI%	Avg. DI%
1	Copper hydroxide 50WG Dodine 400SC	12.0 E	13.05 E	15.85 E
2	Copper hydroxide 50WG Captan 80WG	19.13 D	20.25 D	22.5 D
3	Copper hydroxide 50WG Mancozeb 80WP	22.38 B	24.28 B	26.13 B
4	Copper hydroxide 50WG Tebuconazole 250EW Captan 80WG	20.13 CD	22.13 C	23.63 CD
5	Copper hydroxide 50WG Propineb 70WP Difenconazole 250EC	20.75 C	23.1 BC	24 CD
6	Copper hydroxide 50WG Trifloxystrobin 50WG Chlorothalonil 720SC	20.93 C	22.38 C	23.63 CD
7	Copper hydroxide 50WG Cyprodinil 50WG Dithianon 700WG	20.63 C	22.15 C	24.08 C
8	Control	29.5 A	30.25 A	30.75 A
Sum		165.43	177.58	190.55
Average (Avg)		20.68	22.20	23.82
SMD		1.2794	1.5182	1.5015

The average disease index values are from four replications. The average disease index (DI %) values presented by the same letter do not statistically differ from themselves as per Tukey-Kramer HSD test at level of 5%.

The assessment results for apple scab disease in the fruits for the susceptible Starking cultivar involved in this study during 2017 are presented on table 6. The disease index DI% varies from 6.5%, 7.88% and 8.98% listed in class E respectively class D as per Tukey-Kramer HSD test for the first treatment program with the same fungicides such as Copper hydroxide and Dodine but with different treatment periods comparing with the second treatment program. The second treatment program is with other combination of fungicides that resulted in class changes, class D and respectively class C in compare to other treatment programs. All other treatment programs resulted with higher disease index values such as program 5 and 6 for RIMpro and Phenological phases period, then program 2, 5 and 6 for local farmers period that have no statistical difference between them. After all, each treatment program was compared with the control variant.

The one-way analysis of variance (ANOVA) for assessment of the Apple scab disease index (DI %) on the leaves, presented on table 7 shows the statistically proven differences between the treatment programs realized in all treatment periods. This can be verified from the factual F values for all leaf treatments by: RIMpro period is 311.1**, Phenological phase's period is 230.3** and Local farmer period is 169.4** which resulted to be higher than theoretical F values as per Fisher's table values for the two levels of probability, respectively for P = 0.05 and P = 0.01. From the comparison of factual F values of the repetitions for all leaf treatments realized in RIMpro period (0.182ns), Phenological phases period (1.009ns) and Local farmer period (0.33ns) with

those from Fisher's table values, it resulted that repetitions do not provide statistically proven differences for both levels of authenticity.

Table 6. Disease Index (DI %) on fruits of apple cv. Starking analyzed on Sept. 2017

Treatment Programs		Treatment Periods		
		RIMpro	Phenological phases	Local farmer
		Avg. DI%	Avg. DI%	Avg. DI%
1	Copper hydroxide 50WG Dodine 400SC	6.5 E	7.88 E	8.98 D
2	Copper hydroxide 50WG Captan 80WG	10.5 D	11.75 D	12.95 C
3	Copper hydroxide 50WG Mancozeb 80WP	12.43 B	13.75 B	14.93 B
4	Copper hydroxide 50WG Tebuconazole 250EW Captan 80WG	11.78 BC	12.98 BC	13.75 BC
5	Copper hydroxide 50WG Propineb 70WP Difencconazole 250EC	11.28 CD	12.50 CD	13.4 C
6	Copper hydroxide 50WG Trifloxystrobin 50WG Chlorothalonil 720SC	11.10 CD	12.53 CD	14.03 BC
7	Copper hydroxide 50WG Cyprodinil 50WG Dithianon 700WG	11.5 BCD	12.68 C	13.50 C
8	Control	18.4 A	19.2 A	20.95 A
Sum		165.43	93.48	103.25
Average (Avg)		20.68	11.68	12.91
SMD		1.2794	1.0522	0.9083

The average disease index values are from four replications. The average disease index (DI %) values presented by the same letter do not statistically differ from themselves as per Tukey-Kramer HSD test at level of 5%.

Also, based on one-way analysis of variance(ANOVA) for Apple scab disease assessment on the fruits of the same apple trees, it is shown that there are statistically significant differences between the fungicide treatments performed in all three treatment periods. This is verified from the factual F values for the fruit treatments in RIMpro period (230.3**), Phenological phase's period (262.47**) and in Local farmer period (169.4**) which resulted to be higher than theoretical F values as per Fisher's table values for two levels of authenticity of $P = 0.05$ and $P = 0.01$. From the comparison of factual F values of the repetitions for all fruit treatments in RIMpro period (2.238ns), Phenological phase's periods (0.707ns) and Local farmer periods (0.686ns) with those from Fisher's table values, it resulted that repetitions do not provide statistically significant differences for both levels of probability.

Table 4. One-way analysis of variance (ANOVA) for apple scab disease index (DI %) for eight programs and three treatment periods

Treatment periods	Organ	Sources of Variation	DF	SS	MS	F Values		
						Factual	Theoretical	
							95%	99%
RIMpro (cloud system)	Leaf	Treat. Programs	7	635.2	90.74	311.9**	2.48	3.64
		Repetitions	3	0.158	0.053	0.182ns	3.07	4.87
		Error	21	6.109	0.291	-	-	-
		Variation total	31	641.5	-	-	-	-
	Fruit	Treat. Programs	7	297.9	42.56	230.3**	2.48	3.64
		Repetitions	3	1.241	0.414	2.238ns	3.07	4.87
		Error	21	3.882	0.185	-	-	-
		Variation total	31	303	-	-	-	-
Phenological Phases (cv. Starking)	Leaf	Treat. Programs	7	629.9	89.99	219.70**	2.48	3.64
		Repetitions	3	1.241	0.414	1.009ns	3.07	4.87
		Error	21	8.602	0.41	-	-	-
		Variation total	31	639.8	-	-	-	-
	Fruit	Treat. Programs	7	269.4	38.48	262.47**	2.48	3.64
		Repetitions	3	0.311	0.104	0.7076ns	3.07	4.87
		Error	21	3.079	0.147	-	-	-
		Variation total	31	272.8	-	-	-	-
Local Farmer	Leaf	Treat. Programs	7	475.1	67.8713	169.4**	2.48	3.64
		Repetitions	3	0.396	0.13208	0.33ns	3.07	4.87
		Error	21	8.414	0.40065	-	-	-
		Variation total	31	483.9	-	-	-	-
	Fruit	Treat. Programs	7	304.6	43.52	144.7**	2.48	3.64
		Repetitions	3	0.618	0.206	0.686ns	3.07	4.87
		Error	21	6.314	0.301	-	-	-
		Variation total	31	311.6	-	-	-	-

**Significant at a level of 1% of probability ($P < 0.01$); *Significant at a level of 5% of probability ($0.01 < P < 0.05$); ns: Non-significant ($P > 0.05$).

The two-way analysis of variance (ANOVA) for the assessment of the Apple scab disease index (DI%) on the leaves presented on table 8 shows that effects of factor A (treatment periods) and effects of factor B (treatment programs) are statistically significant for high level of authenticity $P = 0.01$. This can be verified from the factual F values for factor A (228.0671**) and factor B (712.3186**) which are bigger than theoretical F values as per Fisher's table for two levels of probability, respectively for $P = 0.05$ and $P = 0.01$. The effects of interaction between both factors $A \times B$ (2.9808**) also resulted higher than theoretical F values as per Fisher's table for both levels of authenticity.

From the comparison of factual F values of the repetitions for all leaf treatments in RIMpro period (0.182ns), Phenological phase's period (1.009ns) and in Local farmer period (0.33ns) with those from Fisher's table values, it resulted that repetitions do not provide statistically significant differences for the two levels of authenticity.

The effects of factor A (treatment periods) and effects of factor B (treatment programs) for the treatments performed on fruits are statistically significant with high level of authenticity $P = 0.01$ and this is verified from the factual F values for factor A which is valued at 210.4181** and the factor B valued at 579.0756** which resulted to be higher than theoretical F values as per Fisher's table for both levels of probability.

The effects of the interaction between both factors A×B are valued at 0.7908ns which resulted to be lower than theoretical F values as per Fisher's table for both levels of probability, meaning that interaction of these factors practically had no effects to protect the apple Starking fruits from *V. inaequalis* infections.

Table 5. Two factorial analysis of variance (ANOVA) for apple scab disease index (DI %) for eight programs and three treatment periods

Organ	Sources of Variation	DF	SS	MS	F Values		
					Factual	Theoretical	
						95%	99%
Leaf	Treatment periods (A)	2	157.87313	78.93656	228.0671**	3.31	4.92
	Treatment Programs (B)	7	1725.7896	246.54137	712.3186**	2.14	2.91
	Interaction A×B	14	14.44354	1.03168	2.9808**	1.82	2.35
	Treatments	23	1898.1063	82.52636	238.4389**	1.7	2.09
	Error	72	24.92	0.34611	-	-	-
	Total	95	1923.0263	-	-	-	-
Fruit	Treatment periods (A)	2	90.27521	45.1376	210.4181**	3.31	4.92
	Treatment Programs (B)	7	869.53833	124.21976	579.0756**	2.14	2.91
	Interaction A×B	14	2.37479	0.16963	0.7908 ns	1.82	2.35
	Treatments	23	962.18833	41.83428	195.0190**	1.7	2.09
	Error	72	15.445	0.21451	-	-	-
	Total	95	977.6333	-	-	-	-

**Significant at a level of 1% of probability ($P < 0.01$); *Significant at a level of 5% of probability ($0.01 < P < 0.05$); ns: Non-significant ($P > = 0.05$).

4. Conclusion

Among the all treatment programs, from all treatment periods, it appeared that the first treatment program which was consisted with fungicides such as Copper hydroxide 50WG and especially Dodine (Syllit 400SC) had the lowest disease index and provided the best protection from *Venturia* infection. This program was realized based on the period determined by decision support system RIMpro which was provided in the form of warnings for *Venturia* infections to the growers that were connected with this cloud platform.

The first treatment program had the lowest number of application in compare to other programs and therefore had less financial and environmental impact.

Although, the second treatment program realized on the period as per six phenological phases can be seriously considered to be used by the apple growers that do not have the possibility to be connected and to interact with the decision support system RIMpro or any other cloud warning system.

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