

## Managing the scab on apple leaves by combining some treatment programs with application periods

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### Abstract

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The apple scab disease which is caused by the fungus of *Venturia inaequalis* is a continuous problem to the apple growers. The infection requires immediate and continuous treatment in order to control the disease. Nowdays there are several fungicides for treatment of apple scab with different application time. The objective of this study was to identify the optimal action threshold and combining it with some treatment programs for managing the scab disease. For this reason eight treatment programs were created consisting with several fungicides and were realized in three different treatment periods within same year. This research is conducted in one experimental orchard from 2015 to 2017. The disease infection levels were checked on 2400 leaves from 98 apple trees. Based on analyzed disease index the treatment times and variants were compared with each other to results with the best combination of chemicals and application time frame for disease management.

**Keywords:** apple scab; *Venturia inaequalis*; infection; index; treatment

**Abbreviations:** IPM – integrated pest management, DI – disease index, RIMpro – relative infection measure program, DF – degrees of freedom, SS – sum of squares, MS – mean square

### Introduction

In the region of Gjilan and overall in country of Kosovo, one of the major fruit that is cultivated in commercial orchards is the apple fruit. The structure of main cultivars is consisted from Starking, Golden Delicious, Idaret, Granny Smith, Topaz and Gala. Other cultivars such as Braeburn, Jonagold and Fuji continue to be planted rapidly. This crop almost represents 65% of the total number of fruit cultivars. In terms of susceptibility to the pests and infections, the apple Starking cultivar is very sensitive. Although the scab disease is well known for decades from literature and cultivation in practise, the management of scab infections remains the challenge for the local growers in the country.

Apple scab manifest at different stages. The primary infection is initiated by the release of ascospores. These spores penetrate the leaf cuticle, causing the formation of a flat mycelium between the cuticle and the epidermal cell walls (Delalieux et al., 2005). Apple scab, caused by the fungus *Venturia inaequalis* (Cooke.) Wint., is a yearly threat to apple production (Carisse et al., 2009). This pathogen is virulent on all green herbaceous organs of the apple tree. The *V. inaequalis* pathogen may infect and colonize the sepals, leaves, fruits, petioles, blossoms and even twigs of the tree, with symptoms commonly observed on the leaves and fruits. Olive green spots, or lesions, first appear on the leaves soon after bud break. The lesions begin on the underside of the leaf, but are more conspicuous and will be seen on the upper surface (Jamar, 2011). Once the leaves open, the up-

per 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-coloured and velvety due to production of asexual spores (conidia). Lesions that are formed on young leaves may be quite large, some more than 1cm in diameter. Lesions that form 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 and Hartman, 2000). As the fungus progresses the entire leaf appears a 'dirty olive' and falls to the ground (Jamar, 2011). Later in the spring, these primary infections produce secondary type of spores named conidias which infect other leaves and fruits. These secondary infections may continue throughout the growing season during wet periods. The apple scab fungus itself does not "kill" the

tree, but scab infection results in leaf and fruit loss and many susceptible trees are severely defoliated by mid-summer. If the disease is not controlled, over 80% of fruits of susceptible cultivars can be damaged. Depending on the risk of 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 and Gadoury, 1989; Stensvand et al., 1998).

The overall intent of this research was to originate one treatment variant with available plant protection products in the market and finding the optimal action threshold to realize the fungicide application for managing primary infections of apple scab. If the primary spore infections are controlled successfully in the early spring then the secondary conidia infections could be in low level throughout the growing season. Depending on the climate conditions, especially in the

**Table 1. Treatment programs and dates of application for three years**

Program	Plant protection product	Active substance	Concentration	Activity	FRAC Code	Volume per 16 L of water	Application dates 2015	Application dates 2016	Application dates 2017
1	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40.0 g	17.04	21.04	04.04
	2. Syllit	Dodine 400SC	400 g/Lit	Contact	U12	40.0 ml	27.04, 10.05, 25.05, 10.06	01.05, 15.05, 01.06, 16.06	22.04, 15.05, 31.05, 14.06
2	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40.0 g	13.04	16.03	20.03
	2. Captan	Captan 80WG	800 g/kg	Contact	M04	24.0 g	10.05, 25.05, 08.06, 07.07, 18.08	15.05, 03.06, 18.06, 07.07, 25.08	13.05, 01.06, 16.06, 05.07, 30.08
3	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40 g	10.03	05.03	12.03
	2. Mankosav	Mancozeb 80WP	800 g/kg	Contact	M03	32.0 g	13.04, 25.04, 11.05, 27.05, 13.06, 10.07, 25.08	25.03, 20.04, 10.05, 30.05, 15.06, 05.07, 28.08	20.03, 15.04, 08.05, 28.05, 15.06, 05.07, 15.08
4	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40.0 g	10.03	05.03	01.03
	2. Folicur	Tebuconazole 250EW	250g/Lit	Systemic	3	12.0 ml	13.04, 03.05	25.03, 03.05	20.03, 02.05
	3. Captan	Captan 80WG	800 g/kg	Contact	M04	24.0 g	25.05, 01.06, 05.07	01.06, 12.06, 01.07	04.06, 16.06, 05.07
5	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40.0 g	10.03	05.03	01.03
	2. Antracol	Propineb 70WP	700 g/kg	Contact	M03	32.0 g	10.05, 25.05	15.05, 01.06	13.04, 01.06
	3. Score	Difenconazole 250EC	250 g/Lit	Systemic	3	2.40 ml	01.06, 07.07, 27.08	16.06, 07.07, 30.08	15.06, 04.07, 25.08
6	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40.0 g	10.03	05.03	01.03
	2. Zato	Trifloxystrobin 50WG	500 g/kg	Systemic	11	1.60 g	13.04, 03.05	25.03, 03.05	20.03, 05.05
	3. Daconil	Chlorothalonil 720SC	720 ml/Lit	Contact	M05	24.0 ml	01.06, 07.07, 27.08	01.06, 16.06, 05.07	03.06, 19.06, 07.07
7	1. Champion	Copper hydroxide 50WG	500 g/kg	Contact	M01	40.0 g	10.03	05.03	01.03
	2. Chorus	Cyprodinil 50WG	500 g/kg	Systemic	9	8.0 g	13.04, 03.05	25.03, 03.05	20.03, 05.05
	3. Daneel	Dithianon 700WDG	700 g/kg	Systemic	M09	8.0 g	01.06, 07.07, 27.08	01.06, 16.06, 05.07	03.06, 19.06, 07.07
8	Control	No treatments	-	-	-	-	-	-	-

seasons with low amount of rainfall and with temperature increment by the beginning of the summer, conidia infections may need very low quantitative treatment.

The first objective of this research was to establish an optimal action threshold for initiation of the spring chemical treatments. The second objective was to combine several chemicals that would perfectly fit in the originated action threshold.

This research is meant to provide useful information for the apple growers and extension services for managing the apple scab disease in the cultivation zone which was subject to this research.

## Materials and Methods

This research has been carried out during three years 2015-2017 in Zhegra in the region of Gjilan, Kosovo. The experimental orchard is mainly in cultivation with apple cv. Starking but it has other apple cultivars as well. The format of experiment is two factorial randomized blocks with four replications. The experiment is modelled with Factor A for treatment period that has three levels: a) RIMpro action threshold (relative infection measure program); b) phenological phases' action threshold, and c) local farmers' action threshold.

The RIMpro program as an decision support system (DSS) is developed in Netherlands and provides warnings for infection timeliness to the farmers and researchers all over the Europe that are subscribed and connected online. The phenological phases' action threshold are the apple growth stages and for this research only six phases from the BBCH Scale are used: 10, 67-69, 71, 72, 74 and 85-87 (Meier, 2001).

The second effect factor (B) has eight treatment variants. The variants have by two to three fungicides in their combination as shown in Table 1. The product volumes were prepared and mixed as per manufacturer's recommendation on the product label.

**Table 2. Standard area diagram (SAD) field key for apple scab infection assessment**

Leaf						
Category	0	1	2	3	4	5
Intensity level	Nothing noticed	Light intensity	Medium intensity	Strong intensity	Very strong intensity	Destructive intensity
Infection level	0% leaf surface infected	0.1 – 10% leaf surface infected	10.1 – 25% leaf surface infected	25.1 – 50% leaf surface infected	50.1 – 75% leaf surface infected	> 75% leaf surface infected

The third effect factor (C) is the treatment year also in three levels: 2015, 2016 and 2017. The disease severity was determined by rating the scab. Disease severity is a measure of the amount of disease per sampling unit (Nutter et al., 2006).

On the 22 July of every research year, there were randomly picked up 25 leaves (all sides of the tree) from the randomized block trees for assessment. In the laboratory, 2400 leaves were analysed for the disease index, which were taken from 98 apple trees. For each treatment program, the disease infection level was checked based on the leaf surface area infected by the pathogen of *V. inaequalis*.

The infection assessment was defined with field key and then classified in the categories based on Standard Area Diagram (SAD). The six SAD categories are presented in percentage from 0% to 75% of the leaf surface infected area as shown in Table 2 (Hasani, 2005). In order to make sure the infection assessment based on SAD was going accurately, Leaf Doctor Software was used (Pethybridge and Nelson, 2015) to compare some of the infected leaves. The disease index (severity) was calculated through pondered average with McKinney's index (McKinney and Davis, 1925) later modified by Cooke et al. (2006):

$$I = \frac{\sum(n_i \times k_i)}{N \times K} \times 100,$$

where I = disease index;  $n_i$  = number of leaves in respective category;  $k_i$  = number of each category; N = total number of leaves analysed; K = total number of categories.

The trees in randomized block and other trees in the orchard were treated also with other plant protection products for preventive measures against other fungal diseases or pests besides the above fungicides, which were used especially for the managing of the apple scab disease. In addition, other agro technical measures are performed for orchard management.

The weather conditions in the orchard are measured and collected by weather station model i-Metos 2® which has

configured these parameters: temperature ( $^{\circ}\text{C}$ ), relative humidity (RH%), precipitation (mm), dew point ( $^{\circ}\text{C}$ ) and two leaf wetness sensors that provide the duration period of the leaf moisture in minutes per 24 hours. One sensor is for the leaves inside the tree wreath and other sensor is for peripheral leaves. This weather station was set up in the middle of orchard, 2 m above the ground. This station is produced and configured by Pessl Instruments from Austria and monitored by FieldClimate<sup>®</sup> platform from the same inventor.

The ascospores trapping was performed with one spore-trap which is made from the glass and wood material (Ostry and Nicholls, 1982) and was set up in the orchard centre. Later, the glass slides which had one thin layer of vaseline were observed in the microscope model B120C-E1 Am-Scope<sup>®</sup> equipped with digital camera to identify and to make photos of the captured ascospores in the early spring from the glass slides. The other verification was with visual inspection aiming to find and evidence the first conidia scabby spots or lesions on the infected apple leaves.

The fungicide application was performed with Villager<sup>®</sup> spraying pump, model VBS with volume capacity of 16 liters and with spraying pressure 2.6-4.0 atm.

The statistical data analysis for this research respectively

the averages, variance and standard deviation are calculated with statistical program Assistat version 7.7 and the comparison of means for disease index (DI) is completed using the Dunnet's test for two levels of probability  $P = 0.05$  and  $P = 0.01$  with JMP<sup>®</sup> 14.0 program from SAS 2009.

## Results and Discussions

The weather conditions such as temperature, precipitation, relative humidity, dew point and leaf wetness duration for three years, basically for the seasonal months that apple scab primary and secondary infections are mostly developed are measured on the orchard as presented in Table 3.

The natural conditions for the development of primary infection from the ascospores and secondary infection from the conidia of fungus *V. inaequalis* in the apple orchard were optimal. The three-month period April-May-June of each year had numerous rainfalls and favourable temperatures and during three years of this research, we had the development of the apple scab infections. The assessment results for disease index on leaves for Starkings cultivar, during three research years are presented on Table 4.

**Table 3. Monthly weather data for three years (2015, 2016 and 2017) measured on the experimental apple orchard**

Months/ Years	T $^{\circ}\text{C}$ Max.	T $^{\circ}\text{C}$ Avg.	T $^{\circ}\text{C}$ Min.	RH% Max.	RH% Avg.	RH% Min.	Rain (mm)	Dew point T $^{\circ}\text{C}$ Avg.	Leaf wetness inside tree wreath (minute)	Leaf wetness periph- eral leaves (minute)
Febr. 2015	17.2	2.63	-11.3	94.23	74.26	15.2	67.6	-1.2	10045	11120
Febr. 2016	23.5	8.07	-5.6	94.73	73.48	26.1	50.6	2.8	9760	12010
Febr. 2017	19.5	4.08	-6.5	94.15	75.9	21.9	38.8	0.3	5580	13365
March 2015	17.2	8.76	-4.5	98.3	75.85	47.01	104	0.5	19080	19580
March 2016	24.9	8.73	-4.9	97.56	73.96	39.67	123.2	1.8	13210	14935
March 2017	26.9	10.48	-2.8	92.34	63	23.58	21.2	1.1	7115	20915
April 2015	24.9	11.92	-2.1	91.88	59.95	26.29	46	1.2	8490	8785
April 2016	33.2	15.31	-1.3	96.45	62.37	25.11	37	5.4	3965	5290
April 2017	30.4	12.62	-3.5	97.29	63.68	31.23	66.8	2.7	4235	6585
May 2015	33.9	18.38	3.7	96.45	66.81	32.3	37	9.6	10555	9605
May 2016	31.8	14.23	1.5	99.26	75.08	35.62	117.2	8.9	14895	18505
May 2017	32.8	17.37	2.1	100	74.76	31.38	102	9.8	5715	5435
June 2015	33.7	20.3	6.9	96.25	67.97	30.43	56	11.3	11570	8125
June 2016	37.9	22.51	8.6	98.48	69.04	31.62	84.2	13.5	6125	8705
June 2017	39.2	22.27	6.8	100	72.66	25.2	82.4	13.9	5500	4200
July 2015	37.3	24.2	9.1	95.52	58.04	24.16	11	13.1	3420	1545
July 2016	37.5	23.63	8.6	97.97	66.45	29.39	96.8	14.0	5185	4865
July 2017	42.1	25.1	8	97.64	59.37	21.91	19.2	11.8	2145	790
Aug. 2015	36.7	24.35	11.3	91.67	59.07	23.9	44	12.5	5440	4150
Aug. 2016	35.9	21.71	5.7	98.91	71.69	33.06	58.2	13.6	5735	5505
Aug. 2017	42.7	24.73	5.5	97.95	57.4	14.39	54.8	10.6	2545	1945

**Table 4. Disease index (DI%) data analysed on leaves for eight treatment programs realized in three treatment periods for three years**

Treatment programs	Treatment periods																	
	RIMpro time (1)						Phenological phases time (2)						Local farmer time (3)					
	DI % per program/year			Sum	Average	DI % per program/year			Sum	Average	DI % per program/year			Sum	Average			
	2015	2016	2017			2015	2016	2017			2015	2016	2017					
P1	15.50	16.50	12.0	44.0	14.67 C	18.63	20.25	13.05	51.93	17.31 C	21.13	22.93	15.85	59.90	19.97 B			
P2	22.50	22.58	19.13	64.21	21.40 BC	23.25	25.30	20.25	68.80	22.93 BC	25.25	28.65	22.50	76.40	25.47 B			
P3	27.00	27.78	22.38	77.16	25.72 B	28.75	30.10	24.28	83.13	27.71 B	30.00	31.63	26.13	87.75	29.25 AB			
P4	23.00	22.58	20.13	65.71	21.90 BC	25.00	26.88	22.13	74.00	24.67 BC	26.13	27.95	23.63	77.70	25.90 B			
P5	23.50	24.60	20.75	68.85	22.95 BC	26.00	28.45	23.10	77.55	25.85 BC	27.88	29.50	24.00	81.38	27.13 B			
P6	24.25	23.63	20.93	68.81	22.94 BC	25.63	27.50	22.38	75.50	25.17 BC	26.50	27.53	23.63	77.65	25.88 B			
P7	23.25	24.88	20.63	68.76	22.92 BC	25.13	27.35	22.15	74.63	24.88 BC	27.13	29.15	24.08	80.335	26.78 B			
Control	40.25	41.38	29.50	111.13	37.04 A	40.63	42.75	30.25	113.63	37.88 A	40.75	42.38	30.75	113.88	37.98 A			
Average	24.91	25.49	20.68	71.08	23.69	26.63	28.57	22.20	77.39	25.80	28.09	29.96	23.82	81.88	27.29			
LSD = 3.46215 for P = 0.05						LSD = 3.46215 for P = 0.05						LSD = 3.46215 for P = 0.05						

Note: The Tukey-Kramer HSD test at a level of 5% of probability was applied. The averages not connected by the same letter are significantly different.

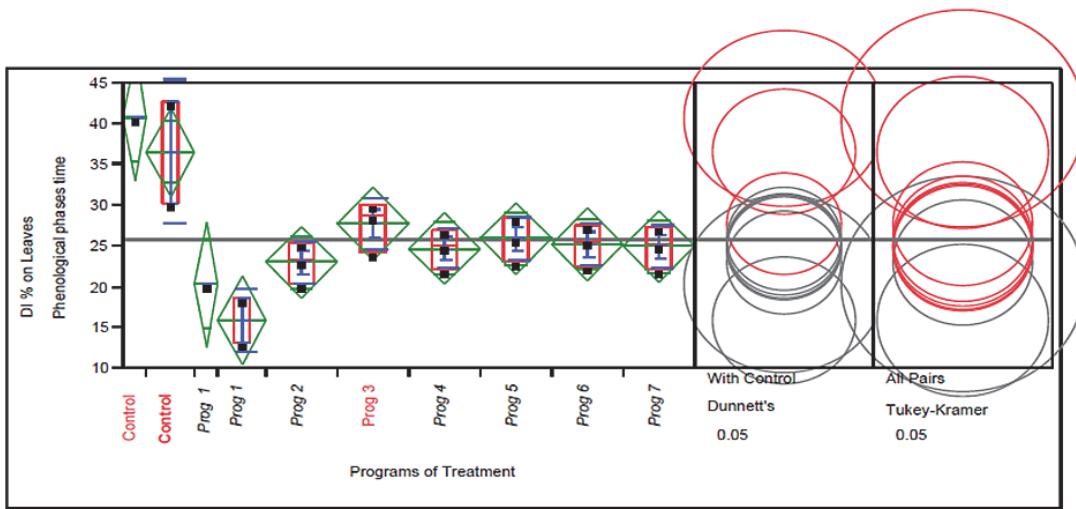
The average disease index (DI%) for the three years as per RIMpro period varies from 14.67% in P1 to 21.40% in P2 and 25.75% in P3. The DI varies in other programs and comparing them all with P8 as the control variant, which has highest index valued at 37.04%. The average disease index for the three years as per phenological phases' period starts with 17.31% in P1 then 22.63% for P2 and increases up to 24.28% in P3. It continues to increase and decrease in other programs as well and finally all programs were compared with P8 as the control variant that has the highest average DI of 37.88%. Also, the average disease index for local farmers'

period for three years starts with 19.97% in P1 then 25.47% for P2 and 29.25% in P3. It varies up and down in other treatment programs and the comparison of all treatment programs with control variant was done so that it resulted that the last one has the highest average DI of 37.98%. The comparison of three annual DI averages per treatment periods shows that RIMpro period for all treatment programs has average DI of 23.69% which is lower than phenological phases' period with DI 25.80% respectively for local farmers' period with DI 27.29%. The comparison of DI averages from all treatment programs realized in three treatment periods during

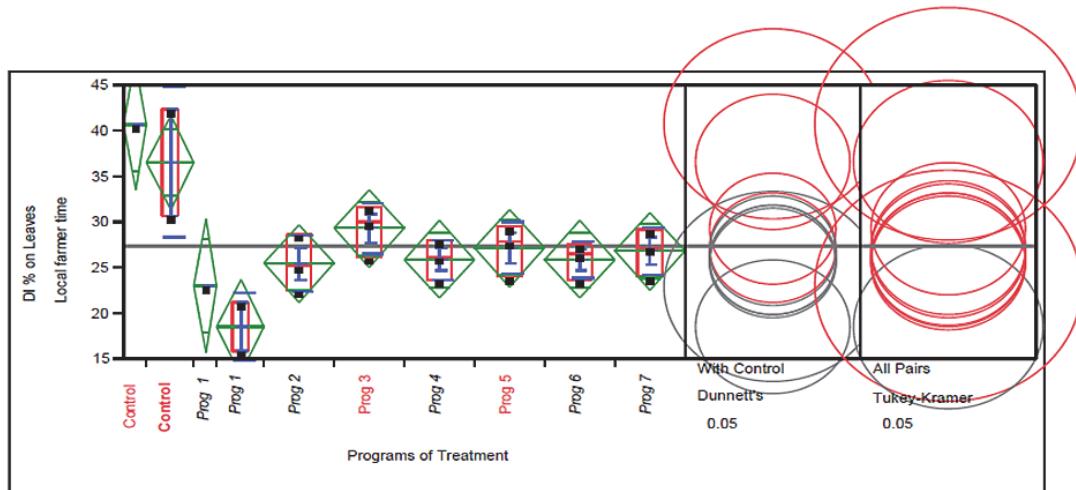
**Table 5. Dispersive analysis of variance (ANOVA) for disease index (DI %) on leaves for eight treatment programs realized in three treatment periods for three years**

Treatment period	Sources of Variation	DF	SS	MS	F Values	
					Factual	Theoretical
						95%
RIMpro	Treatments	7	821.945	117.4208	41.265**	2.76
	Repetitions	2	110.21	55.10527	19.365 **	3.73
	Error	14	39.83	2.845529	—	—
	Variation total	23	971.993	—	—	—
Phenological phases	Treatments	7	696.951	99.56451	44.156**	2.76
	Repetitions	2	170.771	85.38565	37.86**	3.73
	Error	14	31.567	2.254788	—	—
	Variation total	23	899.2898	—	—	—
Local farmer	Treatments	7	536.4163	76.63089	36.773**	2.76
	Repetitions	2	158.7027	79.35135	38.078**	3.73
	Error	14	29.17438	2.083884	—	—
	Variation total	23	724.2933	—	—	—

Note: \*\*P < 0.01, \*P < 0.05, ns: Non-significant P ≥ 0.05



**Fig. 1. Diagram of means diamonds (diamond plot) and comparison circles plot for RIMpro time**



**Fig. 2. Diagram of means diamonds (diamond plot) and comparison circles plot for phenological phases time**

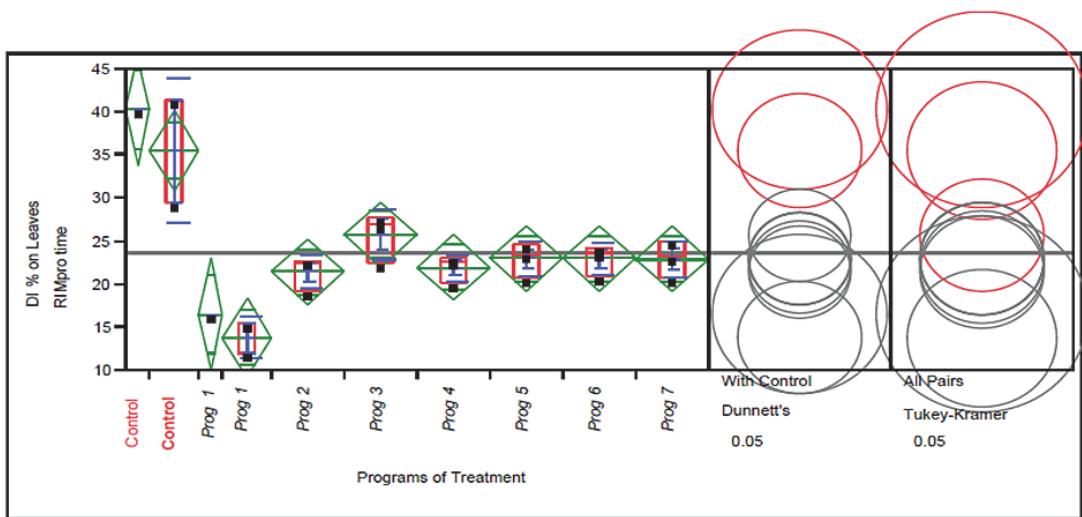
three treatment years results that treatment program one has the lowest DI average than any other program.

The dispersive analysis of variance (ANOVA) for assessment of the apple scab disease index (DI%) on leaves for three years that is presented on Table 5 shows statistically proven differences between the treatment programs for all treatment periods. This can be verified from the factual F value 41.265\*\* for treatments realized in RIMpro period which results to be higher than theoretical F values as per R. Fisher table for two levels of probability, respectively for  $P = 0.05$  is 2.76 and for  $P = 0.01$  is 4.27.

From the comparison of factual F value 19.365\*\* for the

repetitions realized as per RIMpro period with those from R. Fisher table values, it results with the statistically proven differences between the repetitions for two levels of authenticity, for  $P = 0.05$  is 3.73 and for  $P = 0.01$  is 6.51.

The treatment programs performed as per phenological phases' time have the factual F value of 44.156\*\* which statistically proves the significance. This can be verified from the theoretical F values from R. Fisher table for both levels of authenticity, for  $P = 0.05$  is 2.76 and for  $P = 0.01$  is 4.27. From the comparison of factual F values 37.865\*\* from the repetitions performed as per phenological phases' period with those from R. Fisher table, it results with the statistical-



**Fig. 3. Diagram of means diamonds (diamond plot) and comparison circles plot for local farmer's time**

ly proven differences between the repetitions for both levels of authenticity, for  $P = 0.05$  is 3.73 and for  $P = 0.01$  is 6.51.

The treatment programs conducted as per local farmers' period have the factual F value of 36.773\*\* which statistically proves the difference. This can be verified from the theoretical F values from Fisher table for two levels of authenticity, respectively for  $P = 0.05$  is 2.76 and for  $P = 0.01$  is 4.27. From the comparison of repetitions factual F value 38.078\*\* conducted as per local farmers' period with those from Fisher table, it results with statistically proven differences between the repetitions for both levels of probability, for  $P = 0.05$  is 3.73 and for  $P = 0.01$  is 6.51.

The diagram of mean diamonds in Fig. 1 provides the variation between the treatment programs for apple scab disease management on infected leaves as per RIMpro period. The treatment programs with grey circles such programs as 1, 2, 4, 5, 6 and 7 have statistically proven differences for the level of authenticity  $P = 0.05$  as per Dunnet's test and

are below the overall average which in this case for RIMpro period is 23.9%.

The variation between the treatment programs for apple scab disease management on infected leaves as per phenological phases period are provided by the diagram of mean diamonds in Fig. 2. The treatment programs with grey circles respectively the programs 1, 2, 4, 6 and 7 have statistically proven differences for the level of authenticity  $P = 0.05$  as per Dunnet's test and are below the overall average which in this case for phenological phases' period is 25.8%.

The variation between the treatment programs for apple scab disease management on infected leaves as per local farmers' period are provided by the diagram of mean diamonds in Fig. 3. The treatment programs with grey circles such programs as 1, 2, 4 and 6 have statistically proven differences for level of authenticity  $P = 0.05$  as per Dunnet's test and are below the overall average which in this case for phenological phases' period is 27.1%.

**Table 6. Two-Way Analysis of Variance (ANOVA) for disease index (DI%) on leaves for eight treatment programs realized in three treatment periods for three years**

Sources of Variation	DF	SS	MS	F Values		
				Factual	Theoretical	
					95%	99%
Treatment Periods (A)	2	160.96334	80.48317	7.2366**	5.07	6.78
Treatment Programs (B)	7	2034.74484	290.67783	26.1361**	3.03	5.47
Interaction AxB	14	20.50146	1.46439	0.1317ns	0.26	1.45
Treatments	23	2216.21264	96.35707	8.6639**	2.21	3.54
Error	48	533.84227	11.12171	—	—	—
Total	71	2750.05491	—	—	—	—

Note: \*\* $P < 0.01$ , \* $P < 0.05$ , ns: Non-significant  $P \geq 0.05$

**Table 7. Multi factorial Analysis of Variance (MANOVA) for disease index (DI%) on leaves for eight treatment programs realized in three treatment periods for three years**

Sources of Variation	DF	SS	MS	F Values		
				Factual	Theoretical	
					95%	99%
Treatment Periods (A)	2	632.27549	316.13774	129.3799**	3.04	4.71
Treatment Programs (B)	7	8145.80774	1163.68682	476.2409**	2.06	2.70
Treatment Years (C)	2	1729.00340	864.50170	353.7988**	3.04	4.71
Interaction AxB	14	79.85340	5.70381	2.3343**	1.74	2.16
Interaction AxC	4	27.49535	6.87384	2.8131*	2.42	3.40
Interaction BxC	14	383.39215	27.38515	11.2074**	1.74	2.16
Interaction AxBxC	28	19.16076	0.68431	0.2801ns	1.53	1.79
Total treatments	71	11016.98830	155.16885	63.5031**	1.36	1.48
Error	216	527.79250	2.44348	–	–	–
Variation total	287	11544.78080	–	–	–	–

Note: \*\*P < 0.01, \*P < 0.05, ns: Non-significant P ≥ 0.05

The two way analysis of variance (ANOVA) for apple scab disease index (DI%) evaluation on leaves for three years, presented on Table 6, shows statistically proven differences for treatment periods and treatment programs. The effects of factor A (treatment periods) and factor B (treatment program) are statistically proven. This is evident from the factual F value for factor A which is 7.2366\*\* and is higher than both theoretical Fisher table values 5.07 for level of 1% probability and 6.78 for level of 5% probability. The factual F value for factor B is 26.1361\*\* and results higher than theoretical Fisher table values – for P = 0.05 is 3.03 and for P = 0.01 is 5.47. The effects of the interaction between both factors A x B, the factual F value is 0.1317ns and resulted to be lower than theoretical F values as per Fisher tables for both levels of authenticity. Therefore, the interaction of these two factors practically had no effect on scabby leaves.

The multifactorial-factorial analysis of variance (MANOVA) for the apple scab disease index (DI%) assessment on leaves for three years that is presented on Table 7 proved that this disease is influenced by a few factors. This analysis proves that all treatment factors are statistically different.

The effects of factor A, the treatment periods (three different periods of time), have factual F value of 129.3799\*\* which is also much higher than both theoretical F values from Fisher table for both levels of authenticity. Likewise, the effects of factor B, the treatment programs (eight programs with different plant protection products), have factual F value of 476.2409\*\* which is also much higher than both theoretical F values from Fisher table, such values as 2.06 for level of 1% probability and 2.70 for level of 5% probability. The effects of factor C, the treatment years (different climatic conditions on each year), have factual F value of 353.7988\*\*. This value is much higher than both theoreti-

cal F values from Fisher table, such values as 3.04 for level of 1% probability and 4.71 for level of 5% probability. The effects of interaction between two factors AxB = 2.3343\*\* resulted to be higher than theoretical F values from Fishers table for both levels of authenticity. The effects of interaction between the factors A x C = 2.8131\* resulted to be higher than theoretical F values from Fisher table only for one level of authenticity, respectively for P=0.05. The effects of interaction between the factors B x C = 11.2074\*\* also resulted to be higher than theoretical F values from Fisher table for both levels of authenticity. Lastly, the interaction between all treatment factors A x B x C resulted with factual F value of 0.2801ns which is lower than both theoretical F values from R. Fisher table for both levels of authenticity. Therefore, the interaction between all three factors practically had no effect on scabby leaves.

## Conclusion

As per results of disease index assessed on infected apple leaves on each treatment variant in all three treatment periods for three research years and based on statistical comparison between all tested variants with the control variant by using the Dunnett's test and comparison of the tested variants between themselves by using the Tukey Kramer HSD test, it appeared very clearly that plant protection products such as Champion 50WG combined with Syllit 400SC (Table 1) from the first treatment variant/program (P1) has provided the best protection of apple leaves from the scab infections. This treatment program (P1) had also the lowest number of spraying applications comparing to other programs/variants.

Also based on annual and three yearly average disease index assessed on infected apple leaves on each treatment

period, it appeared that DSS RIMpro period has proved to be the best action threshold for all treatment programs execution.

The second treatment period consisting of six phenological phases of the apple cultivar can be seriously considered to be used by the apple growers in this area. Especially those farmers or group of farmers that do not have the possibility to be connected and to interact with the any decision support system online.

The year with the highest apple scab disease index assessed on leaves is 2016 in all treatment periods. The local farmer period has the highest disease index average of 29.96% for year 2016 and the highest disease index average of 27.29% for all three years.

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