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Overview of the decision support system and fruit growth stages to predict the action threshold in order to control the apple scab in Kosovo

Edmond Rexhepi[1], Harallamb Paçe[2], Hekuran Vrapi[3], Arbenita Hasani[4]

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ABSTRACT

The apple scab caused by the fungal pathogen *Venturia inaequalis* (Cooke) G. Winter is a continuous problem for apple farmers in Kosovo. From this disease, the fruits become scabby brown or black spotty, losing their value. The fruit infection from A. scab requires immediate and multi time treatment. In the agricultural market, there are several plant protection products integrated in to various treatment variants provided by chemical manufacturers to control the apple scab infections. The purpose of this study was to identify the best action threshold based on the prediction of the infection risk provided by one decision support system (DSS RIMpro) and based on the empirical point of view by selecting few fruit growth stages from BBCH scale to control this disease in the research zone. Therefore, two different treatment intervals were used and eight treatment variants consisting of several fungicides were created. The research is carried out in one experimental orchard in cultivation with Starking apple cultivar. Based on analysed disease index on infected fruits, the treatment intervals were compared with each other to conclude the best action threshold for Kosovo conditions.

1. Introduction

In the commercial orchards throughout country of Kosovo, one of the major cultivated fruit species is the apple (*Malus domestica* Borkh.). The structure of main cultivars is created from ‘Golden delicious’, ‘Starking’, ‘Gala’ and ‘Granny Smith’. Other cultivars such as ‘Fuji’, ‘Jonagold’ and ‘Braeburn’ continue to be planted. In terms of susceptibility to the diseases and pests, the apple ‘Starking’ cultivar is known as very sensitive among the other cultivars. Taking in to consideration that ‘Starking’ cultivar is being cultivated mostly in the country, the apple scab disease remains the challenge and threat for local growers, especially in wet seasons when the disease can develop faster. This problem causes the fruit cultivation reduction which directly affects the export of the fruit, increases the import to the country which then effects the local growers to remain in solid position in the market.

[1]Edmond Rexhepi
Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment. Tirana, Albania
rexhepiedmond@yahoo.com
[2]Harallamb Paçe
Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment. Tirana, Albania
ha.pace@yahoo.com
[3]Hekuran Vrapi
Agricultural University of Tirana, Department of Plant Protection, Faculty of Agriculture and Environment. Tirana, Albania
hvrapi@gmail.com
[4]Arbenita Hasani
University of Prishtina, Department of Food Technology, Faculty of Agriculture and Veterinary. Prishtina, Kosovo
arbenitahasani@gmail.com

Therefore, the disease needs to be controlled with fungicides. 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 (Mills, 1946; MacHardy and Gadoury, 1989; Stensvand et al., 1998). Important losses occur also due to the development of scab in storage (R. Tomerlin, 1983).

The overall goal of this study was to develop one action threshold with optimal time interval to realize the fungicide treatments to control the apple scab in Kosovo. If the primary infections are not managed successfully on adequate timeframe in the spring, then the secondary infections should remain in high level resulting in the fruit drop during the summer and overall fruit loss in the harvest time. In wet seasons and with temperature increment, the secondary scab infections from conidia will require few fungicidal applications. This study had three main objectives: a) the 1st objective was to start utilizing the DSS in Kosovo plant protection sector; b) the 2nd objective was to analyse the nowadays best decision support system in the market with some selected apple growth stages for predicting the best action threshold to start the treatments to prevent and control the scab disease; c) the 3rd objective was to compare a few treatment variants composed with different fungicides that would perfectly fit in the best action threshold derived from analysis of the treatment intervals. Actual trends in disease management intent to skip the excessive high number of treatments, which involve risks to human health and the environment, and motivate the use of decision support systems (DSSs) (Rossi et al., 2012).

2. Materials and methods

2.1. Location and duration

This research was accomplished in region of Gjilan, country of Kosovo, during three years 2015-2017. The experimental orchard is in 'Starking' cultivation.

2.2. Experimental design

The experimental design is set up in two factorial randomized block with four replications and is formed, as shown in table 1; with Factor A for treatment time interval in two levels: A_1. Phenological phases' threshold; and A_2. RIMpro threshold (relative infection measure program-RIMpro). A_1 - the phenological phases' threshold, consists from apple growth stages from the BBCH Scale (Meier, 2001), as shown in table 2.

A_2 - the RIMpro cloud service is developed by Bio-Fruit-Advies in Netherlands as decision support system (DSS) that provides predictions in forms of warnings for infection periods to the subscribed farmers and researchers all over the Europe and Northern America that are connected and interact with this online platform (figure 1).

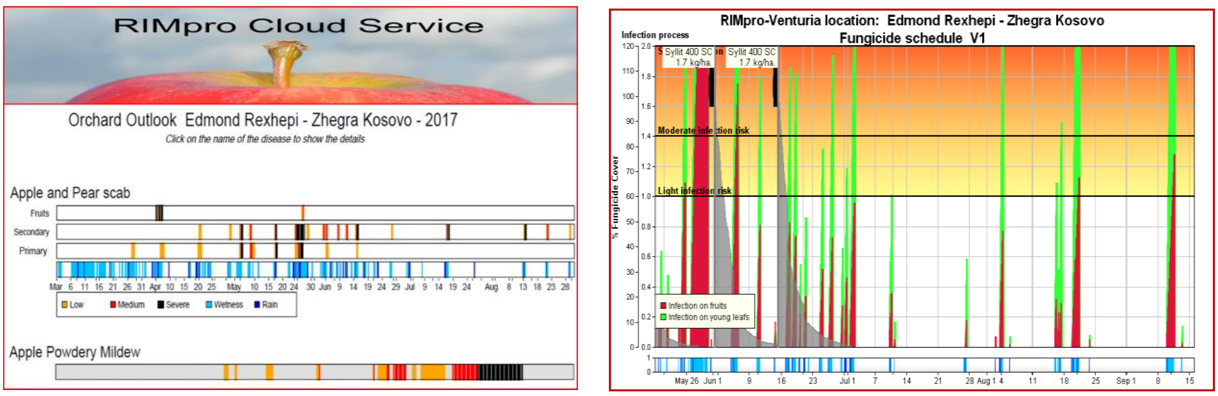


Figure 1. DSS RIMpro: a) platform view; b) infection process

The second effect factor (B) is the treatment variant in eight levels (B_1 - B_8). The levels consist with different fungicides as shown in table 3.

The third effect factor (C) is the treatment year also in three levels: C_1: 2015, C_2: 2016 and C_3: 2017. In this factor the effects of weather conditions dominance were considered for each research year.

Table 1. Treatment intervals and treatment variants for A. scab

YEAR *(Factor C)*		TREATMENT INTERVAL *(Factor A)*	
		A1	A2
C1. 2015		Phenological phases	DSS RIMpro
C2. 2016			
C3. 2017			
TREATMENT VARIANT *(Factor B)*	B1	Copper hydroxide followed by Dodine	
	B2	Copper hydroxide followed by Captan	
	B3	Copper hydroxide followed by Mancozeb	
	B4	Copper hydroxide than Tebuconazole followed by Captan	
	B5	Copper hydroxide followed by Propineb than Difenconazole	
	B6	Copper hydroxide than Trifloxystrobin followed by Chlorothalonil	
	B7	Copper hydroxide followed by Cyprodinil than Dithianon	
	B8	Control (no treatment)	

Table 2. Phenological growth stages and identification keys of pome fruit. *Malus domestica Borkh* (Meier, 2001)

Code	Description
10	Mouse-ear stage: Green leaf tips 10 mm above the bud scales. First leaves separating.
67-69	Flowers fading: majority of petals fallen. End of flowering: all petals fallen.
71	Fruit diameter size up to 10mm; fruit fall after flowering.
72	Fruit diameter size up to 20mm.
74	Fruit diameter up to 40mm; fruit erect.
85-87	Advanced ripening: increase in intensity of cultivar-specific colour. Fruit ripe for picking.

2.3. Sampling

On the 23rd of September in each research year, 10 apple fruits from the randomized block trees were randomly picked for assessment. In the laboratory, 960 fruits were analysed for the disease index which were taken from total of 98 apple trees. For every treatment program, the disease infection level was checked based on the fruit surface area infected by the fungal pathogen *V. inaequalis*.

2.4. Disease assessment

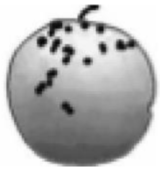
The disease severity was determined by rating the proportion of scabbed surface of the fruit. Disease severity is a measure of the amount of disease per sampling unit (Nutter et al., 2006). Croxall et al. (1952) reported a standard diagram method for rating the scab severity. Lateur and Blazek (2002) reported standard area diagram (SAD) from 1 to 9 scab intensity scale levels. 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 3 (Hasani, 2005).

The disease index was calculated with McKinney's index (McKinney, 1925) which is modified by B.M Cooke (Cooke et al., 2006):

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

I = disease index; ni = number of fruits in respective category; ki = number of each category; N = total number of fruits analysed; K = total number of categories.

Table 3. The Standard Area Diagram (SAD) field key for scab infection assessment on apple fruits

SAD Field Key for *Venturia inaequalis*						
Fruit						
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% fruit surface infected	0.1 - 10% fruit surface infected	10.1 - 25% fruit surface infected	25.1 - 50% fruit surface infected	50.1 - 75% fruit surface infected	>75% fruit surface infected

2.5. Plant Protection Products

The fungicides that were used are shown in table 4. The product volumes were prepared and mixed as per manufacturers' recommendation on the product label. 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 to control the Apple scab. Other regular agro technical actions were performed for orchard management as well.

2.6. Instruments and software

The weather conditions in the orchard and the leaf moisture were monitored and collected by weather station model i-Metos 2 (figure 2). This weather station was set up 2m above the ground in the orchard centre. The leaf moisture data's are gathered by two specific sensors: one sensor was set up inside the apple tree wreath and other sensor was positioned outside the tree wreath. This station is produced and configured by Pessl Instruments GmbH from Austria. The collected data were sent out by the station every 15 minutes to the field-climate platform which is managed by the same inventor/company and after processing in the system, the information or warnings were provided in real time to the researcher/farmer through the same platform. The information was accessible with login in to the field-climate webpage through personal computer and smart phone application.

Figure 2. i-Metos 2, wetness sensor and FieldClimate platform view

The fungicide application was performed with Villager® spraying pump, model VBS with volume capacity of 16 litters and with spraying pressure: 2.6 - 4.0atm.

Table 4. Composition of treatment variants with fungicides

Treatment variant Nr.	Fungicide	Activity	Producer	Treatment variant Nr.	Fungicide	Activity	Producer
1	Champion 50WG	Contact	Nufarm	5	Champion 50WG	Contact	Nufarm
	Syllit 400SC	Contact	Agriphar		Antracol 70WP	Contact	Bayer
2	Champion 50WG	Contact	Nufarm		Score 250EC	Systemic	Syngenta
	Captan 80WG	Contact	Arysta	6	Champion 50WG	Contact	Nufarm
3	Champion 50WG	Contact	Nufarm		Zato 50WG	Systemic	Bayer
	Mancosav 80WP	Contact	Agrosava		Daconil 720SC	Contact	Syngenta
4	Champion 50WG	Contact	Nufarm	7	Champion 50WG	Contact	Nufarm
	Folicur 250EW	Systemic	Bayer		Chorus 50WG	Systemic	Syngenta
	Captan 80WG	Contact	Arysta		Daneel 700WG	Systemic	BASF
8	Control (no treatments)						

2.7. Statistical analysis

For statistical data analysis are used two applications: 1. Assistat® version 7.7 for mean values and standard deviation for treatment variants; 2. JMP® 14.0 is used for Dunnet's and Tukey-Kramer HSD test for comparison of mean values for disease index and diamond/circles plots.

3. Results and Discussion

The assessment results for disease index on apple fruits of Starking cultivar, during three research years are presented on the table 5. The average disease index evaluated on phenological phases' threshold begins with 9.3% in 1st treatment variant which is classified with letter C, then followed by letters BC in 2nd treatment variant with disease index value of 13.0% and up to 21.4% in control variant followed by letter A. The average disease index for DSS RIMpro threshold begins with 7.5% in 1st treatment variant followed by letter C than increases to 11.3% in 2nd variant followed by letter B and slightly increases in other variants but without any significant difference since it is followed by same letter and ends up to 20.4% in control group classified by letter A, as per Tukey-Kramer HSD test. The comparison of three-annual disease index averages from two treatment intervals, shows that RIMpro threshold has average disease index of 12.67% which is lower than phenological phases' threshold with disease index 14.33%.

Table 5. Disease Index (I %) data analysed on 'Starking' fruits for eight treatment variants realized in two treatment intervals during three years.

Treatment variants	Treatment interval							
	Phenological phases threshold				DSS RIMpro threshold			
	DI% per variant/year			Average	DI% per variant/year			Average
	2015	2016	2017		2015	2016	2017	
1	9.53	10.78	7.88	9.3 C	8.25	7.93	6.5	7.5 C
2	13.15	14.30	11.75	13.0 BC	11.90	11.63	10.50	11.3 B
3	15.40	16.50	13.75	15.2 B	14.23	13.50	12.43	13.3 B
4	14.03	15.28	12.98	14.0 B	13.00	12.88	11.78	12.5 B
5	13.30	14.93	12.50	13.5 B	12.18	12.25	11.28	11.9 B
6	13.95	15.75	12.53	14.0 B	12.88	12.75	11.10	12.2 B
7	13.60	15.13	1268	13.8 B	12.38	1.88	11.50	11.9 B
Control	21.75	23.28	19.20	21.4 A	21.38	21.63	18.40	20.4 A
Average	14.34	15.74	12.91	14.33	13.28	13.06	11.69	12.67

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 One-Way Analysis of Variance (ANOVA) for assessment of the scab disease index (I %) on fruits for three years that is presented in table 6, shows statistically proven differences between the fungicide treatment variants in both treatment intervals. The fungicide treatment variants performed in the phenological phases' action threshold period resulted with factual F value of 281.782** which statistically proves the significance comparing to theoretical values from Fisher's table for both levels of probability, for P=0.05 is 2.76 and for P=0.01 is 4.27. The statistical significance is also verified for repetitions factual value with theoretical values for both levels of probability.

The fungicide treatment variants conducted as per DSS RIMpro action threshold resulted with factual F value of 185.94** which is again greater than theoretical values as per Fisher's table for two levels of authenticity, respectively for P=0.05 is 2.76 and for P=0.01 is 4.27. By comparing the repetitions value of this treatment interval with those from Fisher's table, it results with significant difference for both levels of probability.

Table 6. One-Way Analysis of Variance (ANOVA) of disease index (DI %) evaluated on fruits for eight treatment variants performed in two treatment intervals during three years.

Interval	Sources of variation	D.F	S.S	M.S	F Values		
					Factual	Theoretical	
						95%	99%
Phenological phases threshold	Treatments	7	233.5346	33.36208	281.782**	2.76	4.27
	Repetitions	2	32.13578	16.06789	135.712**	3.73	6.51
	Error	14	1.65752	0.118397	-	-	-
	Variation total	23	267.3279	-	-	-	-
RIMpro threshold	Treatments	7	271.714	38.81642	185.94**	2.76	4.27
	Repetitions	2	11.86	5.931838	28.416**	3.73	6.51
	Error	14	2.9224	0.208747	-	-	-
	Variation total	23	286.501	-	-	-	

***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) of the apple scab disease index (I %) evaluation on scabby fruits for three years, presented on table 7, shows the statistical significance for treatment intervals and treatment variants. The effects of factor A (treatment interval) and factor B (treatment variant) are significantly different, based on factual F value of 27.7009** for factor A which is greater than both theoretical Fisher's table values 5.07 for level of 1% of probability and 6.78 for level of 5% of probability. The factual F value for factor B is 58.9703** results to be greater than theoretical Fisher's 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 AxB, the factual F value is 0.1514*ns* and resulted to be lower than theoretical values as per Fisher's table for both levels of authenticity. Therefore, the interaction of these two factors practically had no effect on apple fruits protection.

Table 7. Two-Way Analysis of Variance (ANOVA) of disease index (DI %) evaluated on fruits, for eight treatment variants performed in two treatment intervals during three years.

Sources of variation	D.F	S.S	M.S	F Values		
				Factual	Theoretical	
					95%	99%
Treatment Intervals (A)	2	95.82951	47.91475	27.7009**	5.07	6.78
Treatment Variants (B)	7	714.01443	102.00206	58.9703**	3.03	5.47
Interaction AxB	14	3.66611	0.26187	0.1514*ns*	0.26	1.45
Treatments	23	813.51005	35.37000	20.4484**	2.21	3.54
Error	48	83.02653	1.72972	-	-	-
Total	71	896.53659	-	-	-	-

***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 Multi-factorial Analysis of Variance (M-ANOVA) for A. scab disease index (I %), assessment on 'Starking' fruits for three years, that is presented on table 8, 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 years (different weather conditions), resulted with empirical F value of 303.2630** which is greater than both theoretical values from Fisher's table, such values as 3.04 for the level of 1% probability and 4.71 for the level of 5% probability. The effects of factor B, the treatment intervals (two different action thresholds), resulted with empirical value of 428.7011** which is also greater than both theoretical values from Fisher's table.

The effects of factor C, the fungicide treatment variants (eight variants with different combination of plant protection products), resulted with empirical value that is greater than both theoretical F values for both levels of probability. The effects of interaction between two factors AxB = 19.9297** resulted to be greater than theoretical F values. The effects of interaction between the factors AxC = 1.8507* resulted to be greater than theoretical F value for only one level of probability. The effects of interaction between other two factors BxC = 2.3535** resulted to be higher than theoretical values from Fisher's table for both levels of probability as well.

Lastly, the interaction between all treatment factors AxBxC resulted with empirical value of 0.8490*ns* which is lower than both theoretical Fisher's table values.

Table 8. Multi-factorial Analysis of Variance (MANOVA) for disease index (DI%) evaluated on fruits, for eight treatment variants performed in two treatment intervals during three years.

Sources of variation	D.F	S.S	M.S	F Values		
				Factual	Theoretical	
					95%	99%
Treatment Years (A)	2	272.31194	136.15597	303.2630**	3.04	4.71
Treatment Intervals (B)	2	384.94778	192.47389	428.7011**	3.04	4.71
Treatment Variants (C)	7	2858.19135	408.31305	909.4441**	2.06	2.70
Interaction AxB	4	35.79139	8.94785	19.9297**	2.42	3.40
Interaction AxC	14	11.63250	0.83089	1.8507*	1.74	2.16
Interaction BxC	14	14.79333	1.05667	2.3535**	1.74	2.16
Interaction AxBxC	28	10.67250	0.38116	0.8490*ns*	1.53	1.79
Total treatments	71	3588.34080	50.54001	112.5688**	1.36	1.48
Error	216	96.97750	0.44897	-	-	-
Variation total	287	3685.31830	-	-	-	-

***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 pairwise comparison between the fungicide treatment variants (programs of treatment) with the control group as per phenological phases' action threshold, are presented by the diagram of diamonds plot in figure 3. The fungicide treatment variants with grey circles and italicized variable labels, respectively the programs from 1 to 7 are significantly different from the control group, for the level of probability P = 0.05, as per Dunnett's test. The means of these treatment variants/programs, except the 3rd program, are below the overall average which in this treatment period is 14.33%. The Tukey-Kramer HSD test pairwise comparison between the treatment variants, shows the significant differences in phenological phases' action threshold.

The diagram of means diamonds in figure 4, provides the pairwise comparison between the fungicide treatment variants/programs with the control group, for scab disease control on infected fruits, as per DSS RIMpro action threshold. The treatment variants with fungicides marked with grey circles and italicized variable labels, as from 1 to 7, are significantly different from the control program, P = 0.05, as per Dunnett's test. The treatment variants from 1st to 7th, except the 3rd program, are below the overall disease index average which in this case for DSS RIMpro action threshold is 12.6%. The 1st fungicide treatment variant has the lowest disease index followed by 2nd and 5th. The pairwise comparisons performed with Tukey-Kramer HSD test shows that there is a significant difference between the treatment variants (programs of treatment).

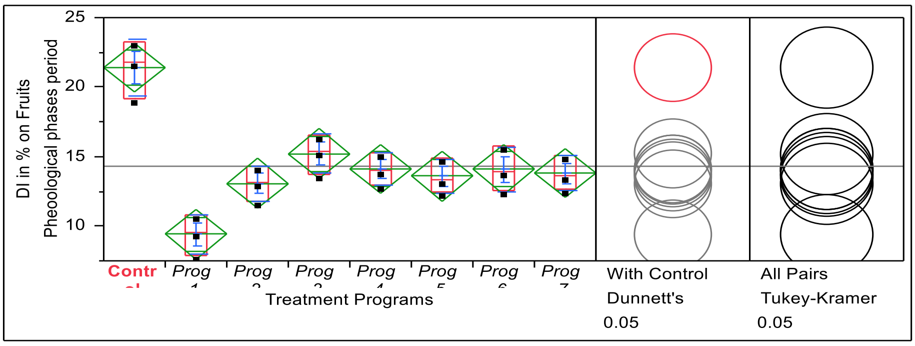


Figure 3. Diagram of means diamonds (diamond plot) and comparison circles plot for Phenological phase's action threshold.

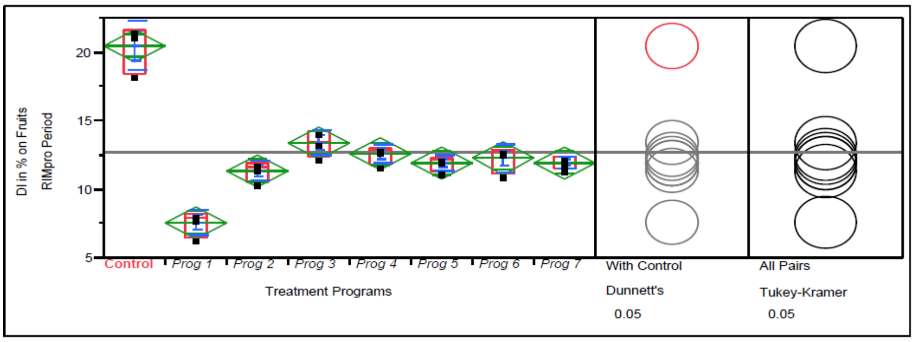


Figure 4. Diagram of means diamonds (diamond plot) and comparison circles plot for DSS RIMpro action threshold.

The top and the bottom points of green diamonds are the confidence intervals (CI), as a supplement to the *p* value with 95% confidence interval for each mean. The green centre line presents the mean. The width of the diamond is proportional to the size of the sample group. The red boxes represent the distribution and the three blue dots per diamond represent the disease index values for three treatment years. The lower/upper horizontal blue lines are for the standard deviation. The grey and red circles represent the means comparison between the treatment variants/programs. The grey circles with *italicized* variable labels are for the variants/programs that are significantly different from the control group. The black horizontal centre line of the diamond and circles plot, is the overall average of the disease index per treatment interval.

Depending on the weather conditions, the apple scab infections happens every season in Kosovo and other countries in region but with different severity level. The issues of A. scab disease are checked from the specialists in the region. The latest studies from the regional countries confirm the scab infections every year. In Serbia, Djordevic et al (2013) tested the tolerance and resistance in few apple cultivars to the scab disease. In Albania, the Skenderasi et al (2013) checked in a couple of times the viability of some fungicides to manage the apple scab based on the infection level. Marku et al (2014) in Albania has evaluated the effectiveness of bicarbonates used alone or combined with horticultural oils to fight the apple scab. The primary cultivar used was 'Starking' and 'Golden delicious' and the scab severity index resulted with close values with this research. Balaz et al., (2017) surveyed the responses of few apple cultivars to apple scab and other diseases under natural infection in Serbia. Rexhepi et al. (2018) in Kosovo has assessed the scab infection level only on apple leaves. No other similar researches of the apple scab control could be found lately for Macedonia and Montenegro. The comparison of findings from similar studies for A. scab severity from other west, central or northern European countries with this research were not performed, since the weather conditions in those sub-climatic zones are slightly different with the research zone in the south-east Europe.

The apple scab fungus behavior and disease severity varies a lot based on the climatic conditions between the climatic zones. In this research, the results showed that based on the comparison of three annual disease index averages presented on table 5, for DSS RIMpro action threshold, the year 2017 had the lowest annual disease index average comparing to the other two research years. This is also the same in other treatment interval, the year 2017 had the lowest annual disease index average. This could be due to the strict regime of fungicide treatment that was performed on trial apple trees and overall in the orchard, continuously for three years. Based on the comparison of disease index averages, from all fungicide treatment variants, realized in both action thresholds and in three treatment years, as presented on table 5, it resulted that: 1st treatment program had the lowest disease index average than any other treatment variant. It seems that this outcome is due to the effects from the fungicide combination of Champion 50WG and Syllit 400SC, which has provided the best fruit protection from the scab infections. The One-Way Analysis of Variance (ANOVA) shows that RIMpro action threshold has the greatest statistical difference comparing to other action threshold. Based on mean comparison in both diamond plots, presented in Figures 3 and 4, it is evidently seen that the first treatment variant which is realized based on the predictions that were provided by DSS RIMpro, had the lowest mean in both treatment intervals.

Based on MANOVA analysis presented on table 8, the effect of interactions between all treatment factors is statistically non-significant. Practically, the small difference of climatic conditions in three treatment years, has not affected the other effects that were derived from treatment variants realized in two action thresholds, and therefore did not change anything significantly regarding the protection of apple fruits from scab infections.

4. Conclusion

Based on the disease index assessed on apple fruits for three years, the second action threshold, a treatment interval predicted by DSS RIMpro and realized through all treatment variants, proved to be the best action threshold. This protection became possible because of the predictions that were received from DSS RIMpro based on weather data collected and provided by weather station from orchard, processed by FieldClimate® platform and sent to RIMpro cloud service for further processing. These predictions from DSS RIMpro were impacted by the information that were provided by the researchers (e.g. green tip date, volumes of used fungicides, etc.). The realization of fungicide spraying on exact time made it possible to create optimal and accurate treatment threshold. Therefore, the DSS RIMpro provided the best treatment interval for all treatment variants execution.

The first treatment interval consisted by six phenological phases of the apple cultivar provided very good action threshold which must be seriously considered to be used by the apple growers in this area. Especially those that do not have the possibility to be connected and to interact with DSS RIMpro or any other decision support system to control the apple scab.

Among the treatment variants, from all treatment intervals, in all three years, it appeared that the first treatment variant, which was composed with fungicides, such as Champion 50WG and Syllit 400SC, had the lowest disease index and provided the best fruit protection from scab infection.

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