RESEARCHES REGARDING FOLIAR AND ORGANIC FERTILIZERS INFLUENCES ON PATHOGENESIS OF *ASCOCHYTA IMPERFECTA* PECK FUNGUS, UNDER THE WEATHER CONDITIONS OF BANAT FIELD

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Abstract

Organic and foliar fertilizers proved to be a great way to supplement the plant's growth stages with micro and macronutrients, helping to improve production and feed quality. Because alfalfa treatment with pesticides against diseases poses serious problems to feed safety and environmental quality, a measure to reduce pathogens virulence would be the correct application and optimal dosage of necessary nutrients.

The influence of foliar (Bionat, Atonik, Cropmax) and organic (manure) fertilizers on the pathogenesis of *Ascochyta imperfect* fungus was tracked at three types of Serbian alfalfa: Novosadanka H – 11, Rasinka and Tisa. Study results show that fertilized plants tolerated the pathogen, susceptibility ranging around 30% compared to the unfertilized witness where the intensity of attacks was of 25.2%. The lowest intensity was registered at the Bionat variant (I = 25%), equal to the virulence of the unfertilized witness (I = 25.2%), emphasizing the indirect role of the bio-stimulator. Novosadanka H – 11 recorded the highest virulence (I = 29.7%). The frequency of affected leaves was higher at fertilized types than at the witness type. These results prove that pathogens' severity is associated with plant nutrition.

Interaction of climatic factors with attack intensity is recorded with a multiple correlation coefficient (R) that exceeds the value of 0.5 (R = 0.526), thus confirming the connection between the above mentioned variables.

Key words: *Ascochyta imperfecta*, foliar fertilizers, organic fertilizers, climatic factors

Alfalfa is attacked by a large number of phyto-pathogenic agents affecting plant virulence, respectively green mass, hay or seeds production, both quantitatively and qualitatively.

Blackleg produced by *Ascochyta imperfecta* fungus often causes defoliation and even death of some parts of the plant. In cold and wet periods, this fungus causes great production losses, affecting feed quality and reducing seeds production. If mowing is delayed, losses are considerable. *Ascochyta imperfecta* fungus is a common disease in alfalfa, especially at those of two or three years old. This pathogen is favored by temperatures between 18 – 21^{0}C at daytime and 13 – 16^0 C at night. At temperatures of 15^{0}C at daytime and 10^{0}C at night, the disease is less severe. High humidity for a longer period of time, hurries pathogenesis by symptoms occurrence and rapid disease expansion (Barbetti, M.J., 1991).

In the 70s, a study conducted in France on alfalfa for feed revealed that the most common leaf pathogens were: *Pseudopeziza medicaginis*, *Ascochyta imperfecta* and *Stemphylium spp.*. During the same period, in Sothern and Eastern Europe, pathogens of economic importance present in alfalfa crops were *Verticillium albo – atrum* first, followed by *Pseudopeziza medicaginis*. *Ascochyta imperfecta* was the third most common pathogen in alfalfa crops (Raynal G., Guy P., 1977). In another study which lasted 10 years (up till 1981), it was proved that *Ascochyta imperfecta* was as frequently found in cultures as *Verticillium albo – atrum*. In 2004, Leyronas, C. et al., indicate that the main pathogens present in alfalfa are: *Pseudopeziza medicaginis*, *Ascochyta imperfecta* and *Uromyces striatus*.

In Romania, losses caused by *Ascochyta imperfecta* can be of 70% in feed production and 30% in seeds production, because this pathogen first infects birds then seeds, where the fungus is located in the superficial layers as a mycelium (Raicu, Cristina, Baciu, Doina, 1978).

Nutrients necessary for plant development are provided by the application of manure, but lately specialized companies produce liquid fertilizer and growth stimulators, directly applied on the alfalfa leaves. These fertilizers complete or replace traditional fertilization processes (Hall, M. H., Stout, R. C., Smiles, W.S., 2002).

Foliar fertilizers proved to be an excellent method to supplement with micro and macronutrients the critical stages of plant growth. Foliar application of micro and macronutrients to alfalfa, alone or in combination with growth regulators, is currently a method for improving feed production and quality. Plants correctly

fertilized better tolerate diseases, due to their virulence and resistance to diseases (Guerena, M., Sullivan, P., 2003).

In the present study, three types of Serbian alfalfa have been monitored, Novosadanka H – 11, Rasinka and Tisa, regarding its behavior to the *Ascochyta imperfecta* fungus attack under different fertilization methods. The influence of fertilizers (growth stimulators and manure) on the fungus virulence was the primary research objective in 2009.

MATERIAL AND METHOD

Pathogenic evolution of *Ascochyta imperfecta* fungus pathogenesis and the relationship with monitored weather factors was pursued in an experiment located within the perimeter of USAMVB Timisoara Teaching Station in June 2009, in different fertilization variants: with organic fertilizers (manure) and growth stimulators (Bionat, Cropmax and Atonik).

In this experiment, a biological material of three types of Serbian alfalfa were used: Novosadanka H – 11, Tisa and Rasinka.

Observations were made during June 2009, on June 15th and 29th. For this period of time, weather data of Timisoara Meteorological Stations were used. The experimental field was located according to the subdivided parcel method, on three repetitions. The size of a parcel was of 5 m^2.

In order to determine the frequency and intensity of *Ascochyta imperfect* fungus attack, samples of fertilized and unfertilized variants (one sample = 10 plants) have been taken for each type of alfalfa. The attack intensity on **stems** and **leaves** was on a scale from 0 to 9. The attack severity or intensity per plant was determined by summing I_{fr} (leaves) and I_t (stem). Both attack frequency and intensity were calculated by using formulas established in phytopathology. Correlation coefficients (r) between weather factors and attack intensity of *Ascochyta imperfecta* pathogens were obtained through statistical calculation (Bravais-Pearson). **SPSS** data a processing program was used.

RESULTS AND DISCUSSIONS

The present study intends to establish the influence of organic fertilizers and growth stimulators on the incidence (aggressiveness) and severity (virulence) of *Ascochyta imperfecta* fungus attack, at three types of Serbian alfalfa, Novosadanka H – 11, Rasinka and Tisa, under the weather conditions of 2009 in Banat Field.

Blackleg caused by *Ascochyta imperfect*a fungus is a disease that frequently occurs in alfalfa crops, being capable of able to produce very significant losses. It may cause premature defoliation and affects plant nutrition, nitrogen fixation capacity also being low. The disease is especially dangerous if it occurs in alfalfa seed crops (Clarke ,Rod, 1999; Ştefan, Camelia, Moisuc, Al., Cotuna, Otilia, 2007; Hulea, Ana, Negru, Al., Severin, V., 1973).

Interaction between plant nutrients and pathogenic agents is very complex and not fully understood. Nutrients lead to plant tolerance and resistance at pathogenic agents; thus properly fertilized plants better tolerate the disease, due to increased virulence and resistance (Dordas Christos, 2008; Guerena M., Sullivan P., 2003).

As plant treatment against disease through the use of pesticide raises serious problems in terms of feed safety and environmental quality, a method for reducing pathogens virulence should be the correct and optimum doses application of necessary nutrients (N, K, P, Mn, Zn, B, Cl, Si). When a plant is attacked by a pathogen, natural defense mechanisms are triggered. Infection causes increased amount of phenolic compounds and flavonoids inhibition both at the infection site and other parts of the plant. These compounds production and transport are largely controlled by plant nutrients. Thus, lack of key nutrients reduces the amount of natural antifungal compounds at the infection site.

Of all nutritive elements, K is the most important in plant disease resistance. Potassium plays an impor tant role in cuticles thickness, which constitutes a physical barrier against infection by pathogens and insects. Dr. Noble Usherwood (1989) reported some results of his researches where he shows that potassium enhances plant health (in 65% of cases), reducing fungal and bacterial infections and infestation by insects and mites.

Potassium fertilization at alfalfa has beneficial impact on the nodes and reduces the severity of common fungal attack that may occur. For best results as regards pathogens, potassium should be applied in doses of 75 kg/ha, while for stimulating the nodes, smaller doses of 61 kg/ha should be applied. Thus pathogen severity is associated with plant nutrition, especially with potassium (Grewal Harsharn Sing, Williams R., 2002). In contrast to potassium, phosphorus involvement in plant resistance is variable.

The types of Serbian alfalfa, Novosadanka H – 11, Rasinka and Tisa, were fertilized with organic fertilizer (manure) in the Autumn of 2007, in November. The experiment being located according to the subdivided parcel method, fertilization was done with different doses of manure, for each type of soil. The manure came from cattle and was applied in doses of 20 t/ha and 40 t/ha. Each variety had unfertilized witness variants. Foliar fertilization with growth

stimulators Bionat, Atonik and Cropmax was done in June, after the second mowing. Doses applied were of 2 l/ha Bionat, 0.5 l/ha Atonik and 1 l/ha Cropmax. The 6th variant was manure and growth stimulators fertilization. Thus, the experiment consisted of seven types of fertilization for each species in the study, including the unfertilized witness.

Regarding the incidence of fungus attack on leaves, it can be noticed that the highest values were recorded in unfertilized variants of the three varieties of alfalfa studied. Variants treated with growth stimulators were more affected by the disease, than the ones fertilized with manure. The most attacked soil was Tisa, followed by Novosadanka H – 11 and Rasinka (*fig. 1*).

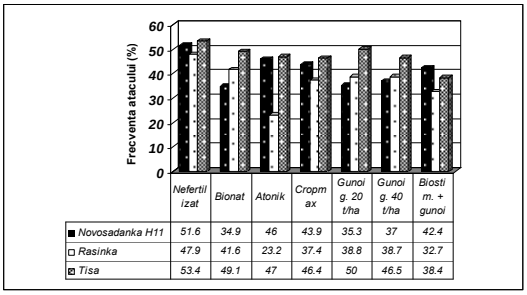

Figure 1 **Graphical representation of *Ascochyta imperfecta* attack frequency in 2009**

Severity or virulence of *Ascochyta imperfecta* fungus infection was different according to the variety and possibility of fertilization.

Analyzing the severity of average infection/experiment, we can notice that virulence values are slightly higher at fertilized variants compared to the unfertilized witness. The highest values occur in variants fertilized with manure applied in the Autumn of 2007 (I% = 30.3 and 32.7%), followed by lower values for variants sprayed with growth stimulators (25% Bionat; 26.4% combined variant; 26.7% Cropmax; 29.3% Atonik). Infection severity at the variant treated with Bionat (I = 25%) is equal to the value registered at the witness variant (25.2%), being the smallest of the whole experiment (*fig. 2*).

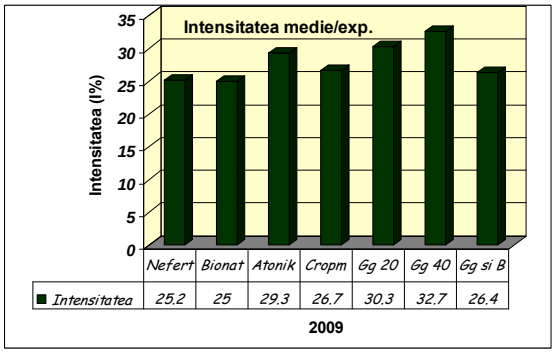

Figure 2 **Graphical representation of average intensity of fertilization variants**

Perhaps Bionat took control of the pathogen by stimulating the formation of physical barriers against the penetration of mycelia hyphae and antifungal compounds, because it is known that this preparation prevents disease occurrence (it increases calcium metabolism, creating a mechanical barrier against fungi, bacterium, microorganisms). Thus, Bionat has also an indirect fungicide role.

The interaction between the weather factors and severity of *Ascochyta imperfecta* fungus infection was established by statistical analysis (Bravais – Pearson correlations and elasticity coefficients). Weather data of June 2009 (temperature, rainfalls, and relative humidity) and average intensity of attack for each fertilization variant (average on experiment) were included in the study – *tab. 1, fig. 2.*

Based on correlation coefficients (*tab. 2*), it can be noted that average temperature has a negative influence on fungus virulence. In the current case, relative humidity and rainfalls act against the fungus growth.

The relationship between the factors included in the present study was analyzed based on the correlation (R) and determination (R Square) coefficients method PEARSON. Following the calculation a relevant model of multiple correlation was obtained. The calculated correlation coefficient is 0.526 (*tab. 3*). The link is low, due to the small number of variants, leading to a lesser degree of data homogeneity. The value of Durbin – Watson coefficient of 2.619, shows that there is a connection between the analyzed factors.

Elasticity coefficients are another way to enhance the comparativeness degree of regression coefficients. Such a coefficient measures with how many percentages the dependent variable is expected to be modified, for a modification with one percentage of the reference independent coefficient, if the other independent variables are kept under control. Elasticity coefficient obtained for the temperature factor demonstrates that disease severity decreases with 0.5858 units, the other factors remaining unchanged due to higher temperature (*tab. 4*). Following the increase of humidity with one percent, attack intensity will grow with 0.796 percentages. Same thing happens for the rainfalls at rainfall increase with one unit, the value of intensity being higher with 0.1586 percentages.

Table 1

Climate data used in statistical processing of research results

No	Average air temperature ^{0} C	Relative humidity UR%	Rainfalls (mm)
1	15.2	81	29.7
2	23.9	61	2.6
3	20.0	60	25.6
4	23.5	61	3.2
5	17.6	87	42.9
6	20.3	84	7.6

Table 2

Simple correlation coefficients derived from statistical calculation

		Intensity	Temperature	Humidity	Rainfalls
Pearson Correlation	Intensity	1,000	-0.133	0.297	0.273
	Temperature	-0.133	1.000	-0.630	-0.766
	Humidity	0.297	-0.630	1.000	0.265
	Rainfalls	0.273	-0.766	0.265	1.000
Sig. (1tailed) probability	Intensity	.	0.388	0.259	0.277
	Temperature	0.388	.	0.065	0.022
	Humidity	0.259	0.065	.	0.283
	Rainfalls	0.277	0.022	0.283	.

Table 3

Multiple correlation model

Model	R	R^2	Sig. F Change	Durbin Watson
1	0.526[a]	0.276	0.775	2.619
a. Predictors: (Constant), Rainfalls, Humidity, Temperature				
b. Dependent Variable: Intensity				

Table 4

Elasticity coefficients derived from statistical calculation

Independent variable	Partial regression coefficient	Average of independent variable	Average of dependent variable	Elasticity coefficient obtained
Temperature	- 0.133	20.0857	27.9429	-0.5858
Humidity	0.297	74.8571	27.9429	0.796
Rainfalls	0.273	16.2286	27.9429	0.1586

CONCLUSIONS

Severity and incidence of *Ascochyta imperfecta* fungus attack were different depending on the type of soil and fertilization variant. Frequency (incidence) of affected leaves registered the highest values at unfertilized variants (F = 47.9% - Rasinka and 53.4% – Tisa). In contrast, variants fertilized with manure (F = 35.3 – 50%) were much more attacked when sprayed with growth stimulators (F = 23.2 – 49.1%).

In the case of infection severity or virulence, differences to the witness were lower. Thus, the most attacked variety in 2009 was **Novosadanka H – 11** (I = 29.7%). Regarding the severity of

average infection/experiment, values were slightly higher at fertilized variants compared to the witness, especially at variants where manure was applied (I = 30.3 and 32.7%). The lowest attack intensity was registered at **Bionat variety** (I = 25%), equal to the unfertilized witness virulence (I = 25.2%), highlighting growth stimulator's role as an indirect fungicide.

Fertilizers' influence on fungus virulence was **positive,** the three soils tolerating all fertilization variants, infection intensity being around 30%, slightly higher that of the witness (I = 25.2%). These results prove that disease severity is associated with plant nutrition.

The interaction of weather factors with attack intensity is supported by the multiple correlation coefficient R, which passes the value of 0.5 (**0.526**), confirming the relationship between the studied variables. **Humidity and rainfalls** were the main weather factors involved in the fungus virulence increase in 2009.

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