

INVESTIGATIONS ON RESISTANCE OF SOME TOBACCO VARIETIES TO *PERONOSPORA TABACINA* ADAM IN THE CORESTA BLUE MOLD COLLABORATIVE EXPERIMENT

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ABSTRACT

Blue Mold Collaborative Experiment includes tobacco varieties from various countries and climate conditions. The aim of investigation was to monitor the intensity of disease attack in a three-years period (2009 - 2011) and to estimate the degree of resistance to the pathogenic fungus *Peronospora tabacina* Adam.

Field trial was set up in randomized blocs with 4 replications. Cultural practices applied in the experiment were in compliance with CORESTA and AERET recommendations for large leaf tobaccos.

The most susceptible variety during the investigation period was Jupiter, while the variety BC1-09-VC1 showed to be tolerant to the disease. The most resistant varieties were Bel 61-10, Chemical Mutant and line V53 CMS F1. The other investigated varieties, among which B2/93 CMS F1, could also be considered as resistant.

Macedonian Virginia and Burley varieties (V53 CMS F1 and B2/93 CMS F1) showed considerably good results in all three years of investigation. These results are of particular importance not only for protection of tobacco from the disease but also for obtaining high yield and good quality of the raw material.

Keywords: Blue Mold, V53 CMS F1, B2/93 CMS F1, degree of infestation, degree of resistance.

ИСПИТУВАЊЕ НА ОТПОРНОСТА НА НЕКОИ СОРТИ ТУТУН СПРЕМА *Peronospora tabacina* Adam ВО ЗАЕДНИЧКИОТ ОПИТ НА CORESTA

Заедничкиот опит на CORESTA за испитување на пламеницата опфаќа тутунски сорти со потекло од разни земји, со разни климатски услови. Целта на ова истражување беше да се следи интензитетот на напад од болеста во текот на тригодишниот период (2009-2011) и да се утврди реакцијата на сортата, односно, да се определи степенот на отпорноста спрема патогенот *Peronospora tabacina* Adam.

Поставен беше полски опит со испитуваните сорти во рандомизиран блок систем, во 4 повторувања. Применувани беа агротехнички операции во склад со насоките на CORESTA и AERET, како и производството на крупнолисни тутуни.

Во тригодишниот период на истражување како осетлива сорта се истакна Jupiter, а како толерантна BC1-09-VC1. На врвот на отпорните сорти се Bel 61-10, Chemical Mutant и линијата V53 ЦМС F1. Како отпорни може да се сметаат и другите испитувани сорти, меѓу кои и B2/93 ЦМС F1.

Македонските сорти од типовите вирџинија (V53 ЦМС F1) и берлеј (B2/93 ЦМС F1) покажаа доста добри резултати во текот на тригодишните истражувања.

Добиените резултати се од особен интерес како за заштитата на тутунот од оваа болест, но и за добивање на тутунска суровина со добар принос, а пред се, квалитет.

Клучни зборови: Blue Mold, V53 ЦМС F1, B2/93 ЦМС F1, интензитет на напад, степен на отпорност.

INTRODUCTION

Blue mold, caused by pathogenic fungus *Peronospora tabacina* Adam, is one of the most harmful diseases on tobacco.

The disease was noticed for the first time in 1891 on cultivated and wild tobacco species in Australia. Its massive attack in 1961 caused serious loss in many tobacco producing countries, including Macedonia. Soon after its outbreak in Europe, it has become economically important disease, primarily because of the importance of tobacco culture and the damages it causes to tobacco yield and quality.

According to Mickovski (1984), climate is one of the major factors for occurrence of blue mold. High amplitudes between day and night temperatures (warm days and cold nights) are especially favorable for disease development. The optimum mean daily temperature for blue mold growth is 20^oC (24^o /16^o C day / night).

Blue mold intensity increases with the increase of relative humidity. Prolonged humid periods are suitable for formation of higher number of spores and for their germination. Another factor that favors occurrence of the disease and spreading of spores is the retention of water drops on leaf surface for at least 1 -3 hours. Therefore, the most suitable for occurrence and development of this disease is the period after heavy rainfalls - when the weather is calm and colder. Vice versa, in conditions of permanent light and aeration, possibilities for disease occurrence and growth are much lower.

The parasite is spread by strong winds, through mass conidia dissemination at wide distances. Therefore, monitoring of the disease in many countries, especially in the neighboring ones, is of particular

importance and is considered as one of the preventive measures of protection.

Variety reaction to the pathogen is another important factor for occurrence of the disease. For this reason, investigation on various degrees of susceptibility and resistance in tobacco varieties is made by CORESTA (Center for coordination of scientific research on tobacco). Collaborative experiment on PTA monitoring has been successfully conducted since 2005, under the auspices of AERET (European Association for monitoring and research of tobacco). Scientific Tobacco Institute -Prilep is participating in this project with line V53 CMS F1 (hybrid) and variety B - 2/93 CMS F1, created in its Genetics and selection Department.

Line V53 CMS F1 was tested in 2002, in Tobacco Institute – Scafati, Italy and based on the results obtained it was included in the project. In 2003 Burley variety B-2/93 CMS F1 was tested and due to the satisfactory results it was also included in the experiment for PTA monitoring. Since then, the above two large-leaf tobacco varieties have continuously been a constituent part of the selection of tobaccos from all over the world in the scope of this international experiment.

During the whole period of investigation, these varieties showed satisfactory resistance to PTA (especially line V53 CMS F1).

The aim of this paper is to present the results of investigations to PTA resistant varieties in 26 countries-participants in this project, which belong to various climatic zones with different environmental conditions, e.g. Macedonia, Germany, USA, Guatemala and others.

MATERIAL AND METHODS

Virginia and Burley varieties and lines originating from France, Germany, Switzerland, USA, etc. were taken as material for investigation. The complete list of tobacco varieties and seed materials included in the experiment can be obtained from the project coordinator. Planting takes place in different time periods, depending on climate conditions of the country where the experiment is carried out.

In R. Macedonia, the experiment was conducted in the field of Scientific Tobacco Institute–Prilep and sowing starts in March. During seedbed production, usual cultural practices were applied (use of herbicides, fertilization, irrigation, regulation of temperature regime, weeding). If conditions were favorable for disease occurrence, preventive protection with contact fungicide and insecticide was made. Soil processing was done in autumn, by plowing to a depth of 30-40 cm. Fertilization was made with NPK fertilizer

(8:22:20), followed by two spring plowings. Prior to transplanting, harrowing was performed for herbicide incorporation in soil.

Transplanting was performed during May or in early June, on alluvial-colluvial soils. The experiment was set up in randomized blocks with four replications. Each replication consisted of 2 rows with 11 plants and planting density was 50x50 cm. During the growing period, tobacco was hoed twice, fertilized with 26% KAN and irrigated when necessary. From protective chemicals, only insecticides were allowed.

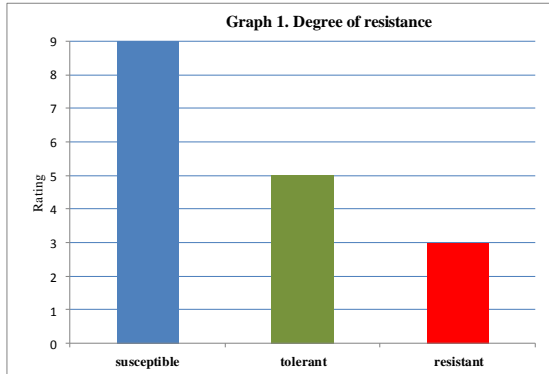
Monitoring (especially on blue mold occurrence) was done on daily basis, from planting to the end of growing season. The intensity of PTA attack is estimated on bottom, middle and top belts, according to CORESTA recommendations, using LTZ Augustenberg scale (Table 1). The degree of resistance is presented in Fig. 1 (Billenkamp and Dongmei, 2011).

Table 1. Scale for assessment of the degree of infestation with *Peronospora tabacina* Adam

Scale	% leaf area damaged*
1	0 - 0,6 %
2	0,6 - 3 %
2	3 - 6 %
3	6 - 12 %
4	12 - 25 %
5	25 - 50 %
6	50 - 75 %
7	75 - 87 %
8	87 - 93 %
9	93 - 97 %
9	97 - 100 %
9	100%

*all PTA symptoms (sporulation, systemic)

Degree of resistance was calculated by the mean value of intensity of PTA attack in the three belts. The varieties were classified into 3 categories: resistant (1 - 2.99), tolerant (3 – 4.99) and susceptible (5-9) (Graph 1).



Growth stage of tobacco varieties was determined by certain morphological

characters (leaf number, flowering percentage) and indicated by numerical code, according to the instructions given by CORESTA Guide No 7.

The list of investigated varieties is presented together with the results of investigation. The Jupiter variety was marked as susceptible to PTA and Bel 61-10 and Chemical Mutant were marked as resistant.

The annual results of the experiment obtained from each country – participant were sent to the project coordinator after the end of the growing season. After processing of data, a report was made with average results for PTA resistance for each variety.

RESULTS AND DISCUSSION

The blue mold disease attacks tobacco in all stages of its growth, from seed to the final harvest (Fig. 1).

Disease symptoms in transplanted tobacco are recognized by the appearance of yellow spots on the leaf. They are localized in the beginning but later their number increases (Fig 2, 3). They often coalesce and occupy most of the leaf area, including leaf veins. Such symptoms can be observed in conditions of high intensity of the attack.



Fig. 1 PTA in seedbeds



Fig. 2 Occurrence of disease

In favorable conditions for disease development, a greyish-blue downy mold appears on lower surfaces of leaves. Actually, it is made of conidiophores with conidia of the parasite fungus. Hence the name of the disease: blue mold (Fig. 4).

All symptoms of the disease are included in the above mentioned scale.

The average results of Blue Mold Collaborative Experiment in the period 2009-2012 are presented in Table 2.



Fig.3 More severe attack of Blue mold



Fig. 4 Blue mold on tobacco leaves

In 2009, the lowest intensity of attack and the best rating was observed in line V53 CMS F1 (Table2)

The highest intensity of attack (5.66) was observed in Jupiter (susceptible check). No results are presented for the varieties Chemical Mutant and HYV 27. In other varieties, the intensity of attack ranged from 1.72 in Bel 61-10 (resistant check) to 3.13 in B-2/93 CMS F1.

According to the scale of resistance (Graph 1), most of the varieties in 2009 were marked as resistant, and the variety B-2/93 CMS F1 as tolerant.

In 2010, intensity of attack was higher in the most varieties. The lowest intensity was observed in Chemical Mutant (1.68) and the highest in Jupiter (6.26).

Varieties BCE/09/VC1, HYV 27, B911, ITB 583, Stella and B2/93 CMS F1 were rated as tolerant, whereas line V53

CMS F1 and varieties ITB 569 and ITB 420 were rated as resistant.

In 2011 the intensity of PTA attack was lower in almost all varieties. Similar to previous year, the highest susceptibility was observed in Jupiter, which achieved the highest intensity - 6.24. The most tolerant variety was BCE-09-VC1, with an intensity of 3.33.

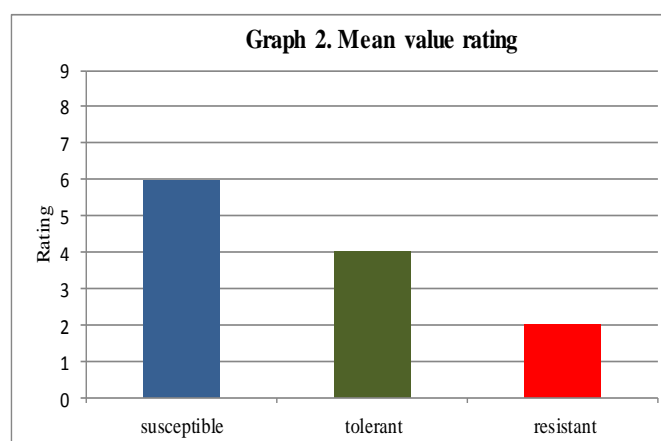
In addition to Bel 61-10 and Chemical Mutant (resistant checks), other varieties that were rated as resistant were ITB 569 (2.12), HYV 27 (2.29), B2/93 CMS F1 (2.31), V53 CMS F1 (2.44), Stella (2.64) and B911 (2.69).

Mean value of the intensity of attack in the varieties of corresponding category and their relation to the degree of resistance is presented in Graph 2. The average intensity of attack ranges from 5.68 in susceptible varieties, 3.05 in tolerant and 2 in resistant ones.

Table 2. Assessment of the varieties reaction to *Peronospora tabacina* Adam

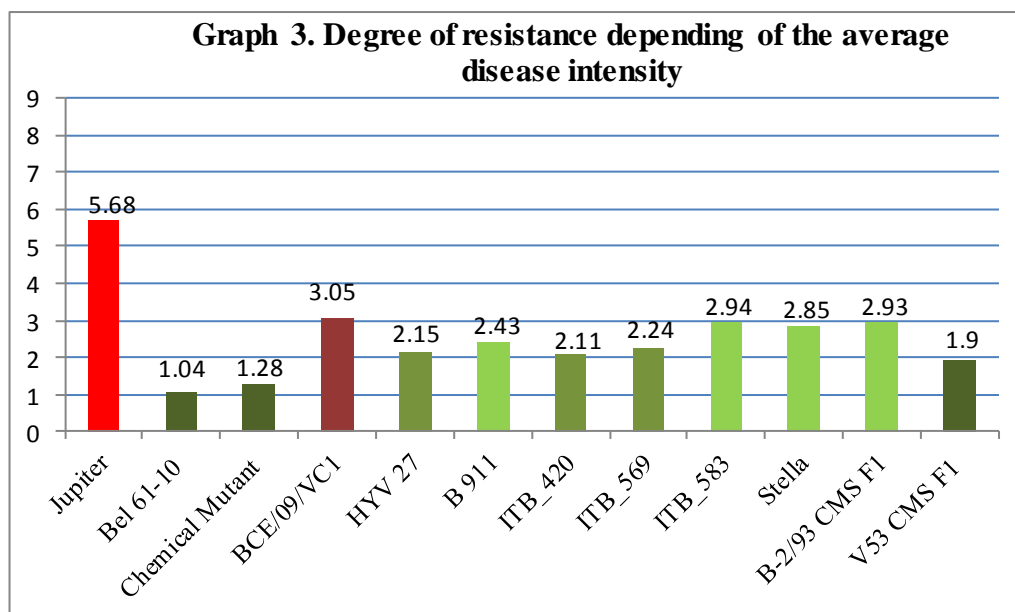
N ^o	Variety	Assessment						Average	Rating
		Year							
		2009	Rating	2010	Rating	2011	Rating		
1	Jupiter	5,66	10	6,26	12	6,24	10	5,68	12
2	Bel 61-10	1,72	2	2,21	4	1,09	1	1,04	1
3	Chemical Mutant			1,68	1	1,49	2	1,28	2
4	BCE/09/VC1	2,92	8	3,77	11	3,33	9	3,05	11
5	HYV 27*Germany			3,06	6	2,29	4	2,15	5
6	B911*Germany	2,53	6	3,10	7	2,69	8	2,43	7
7	ITB_420*France	2,07	4	2,15	3			2,11	4
8	ITB_569*France	2,00	3	2,60	5	2,12	3	2,24	6
9	ITB_583*France	2,74	7	3,14	8			2,94	10
10	Stella *Switzerland	2,44	5	3,48	10	2,64	7	2,85	8
11	B-2/93*FYROM	3,13	9	3,35	9	2,31	5	2,93	9
12	V53*FYROM	1,20	1	2,06	2	2,44	6	1,90	3

*Country of origin of tobacco varieties/lines



The average results reveal the degree of resistance of investigated varieties in the period 2009-2011. The lowest intensity of PTA attack, i.e. the highest degree of resistance was observed in Bel 61-10 (1.04), Chemical Mutant (1.20) and V53 (1.90) (Table 2, Graph 3).

Other varieties that can be considered as resistant are ITB 420 (2.11), HYV 27 (2.15), ITB 569 (2.24), B911 (2.43), Stella (2.85), B2/93 (2.93) and ITB 583 (2.94).



The results of our susceptibility/resistance investigations under conditions of natural infestation are in compliance with those of Marani et al. (1972). They made a crossing between two resistant and one susceptible local variety. F1 and F2 generations and their parents were investigated in a series of experiments in which tobacco seedlings were exposed to severe natural infection with *Peronospora tabacina* Adam.

Gilham et al. (1987) reported that monitoring of blue mold resistance, as well as other traits in tobacco lines obtained by crossing, will allow their further investigations as potentially commercial varieties.

The results of our investigations are in compliance with those of Pejcinovski and Mitrev (2007), who reported that lower disease index indicates higher degree of resistance, and vice versa. Accordingly, varieties Bel 61-10, Chemical Mutant and V53 CMS F1, which showed the lowest disease intensity, showed the highest degree of resistance to the pathogen *Peronospora tabacina* Adam. The highest intensity of attack was observed in Jupiter, due to which it was used as susceptible check.

It can be seen from the ratings that Bel 61-10 and Chemical Mutant (resistant check varieties), are immediately followed by the line V53 CMS F - creation of Tobacco Institute-Prilep. The lowest ranked variety is Jupiter. Variety B2/93 – another creation of Tobacco Institute-Prilep also belongs to the category of resistant varieties (Graph 2).

Considering the fact that Burley tobacco is genetically more susceptible compared to Virginia, it can be concluded that the above rating of B2/93 is quite a good result.

Selection of hybrids with resistance to *Peronospora tabacina* Adam can provide a significant reduction in pesticide use and improvement of safety and quality of tobacco (Tso, 1990). This claim is confirmed by our selection of large-leaf tobaccos with significant resistance to this pathogen.

The above data indicate that Macedonian tobaccos of the types Virginia (V53 CMS F1) and Burley (B2/93 CMS F1) successfully cope with other varieties originating from countries with much longer tradition in selection of large-leaf tobacco.

CONCLUSIONS

-The most severe attack of PTA disease was observed in 2010.

-The most susceptible variety in the three year investigation period was Jupiter.

-In 2009, the lowest intensity of attack was observed in Macedonian line V53 CMS F1 according to which it is considered as PTA resistant variety.

-In 2010, the lowest intensity of attack was observed in Chemical Mutant.

-In 2011, the lowest intensity of the disease was found in varieties Bel 61-10 and Chemical Mutant (resistant checks).

-According to the average results of investigations on severity of PTA infestation, line V53 has the second best rating after the resistant check varieties.

-With regard to PTA resistance, Macedonian large-leaf tobaccos successfully keep pace with countries which have much longer tradition in their production.

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