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**EFFECTIVE CONTROL OF THE TOBACCO SEEDLINGS FROM DAMPING OFF  
DISEASE IN DUAL INFECTION IN BEDS****Biljana Gveroska**University "St. Kliment Ohridski" - Bitola,  
Scientific Tobacco Institute –Prilep,  
Republic of North Macedonia  
e-mail: bgveros@yahoo.com**ABSTRACT**

Damping off disease is caused by pathogenic fungi *Rhizoctonia solani* and *Pythium debarianum*. Its control is very difficult because of similarity of symptoms and use of non compatible fungicides for the target pathogens. The biggest problem is the dual infection which is very often, therefore the use of the proper control is necessary. According these facts, the aim of this study was to determine the most effective way of control. The investigations were carried out in tobacco beds, with two applications of fungicides. They were chosen by instructions, results from artificial inoculation and the previous practice. The most effective control is a fungicide who acts against two pathogens or combination who act separately to each pathogen or simultaneously. The highest effectiveness is achieved by Quadris 25 SC (250g/l azoxystrobin) at 0.15% - 100% effectiveness and Signum 33 WG (267 g/kg boscalid + 67g/kg pyraclostrobin) at 0.1% - 85.05%. Two combinations: Orvego (300g/l ametoctradin + 225g/l dimetomorph) at 0.1% + Manfil 80WP (800 g/kg mancozeb) at 0.25% - 88.64% and Orvego at 0.1% + Signum 33 WG at 0.1% - 84.23% effectiveness also achieved high results. These fungicides are excellent chemical control for damping off disease in dual infection by two pathogens in tobacco seedbeds.

**Keywords:** damping off, fungicides, effectiveness, Quadris 25 SC, Signum 33 WG, Orvego

**ЕФИКАСНА ЗАШТИТА НА ТУТУНСКИОТ РАСАД ОД БОЛЕСТА СЕЧЕЊЕ  
ПРИ ДВОЈНА ИНФЕКЦИЈА ВО ЛЕИ**

Болеста сечење кај тутунскиот расад е предизвикана од патогените габи *Rhizoctonia solani* и *Pythium debarianum*. Нејзината заштита е доста тешка поради сличноста на симптомите и примена на некомпатибилни фунгициди за целните патогени. Најголем проблем е двојната инфекција која е доста честа, поради што примена на правилна заштита е неопходна. Согласно овие факти, целта на ова истражување беше да се утврди најефикасната заштита. Испитувањата беа изведени во тутунски леи. Направени беа две апликации на фунгициди. Тие беа одбрани врз основа на упатствата, резултатите од вештачката инокулација и практичните искуства. Најефикасна заштита претставува комбинација од двата фунгицида кои делуваат одделно кон секој патоген или истовремено. Најголема ефикасност беше постигната со Quadris 25 SC 0.15% (250g/l azoxystrobin) -100% и Signum 33 WG (267 g/kg boscalid + 67g/kg pyraclostrobin) 0.1% - 85.05%.

Двете комбинации: Orvego (300g/l ametoctradin + 225g/l dimetomorph) 0.1% + Manfil 80WP (800 g/kg mancozeb) 0.25% -88.64% и Orvego 0.1% + Signum 33 WG 0.1% - 84.23% исто така постигнаа добри резултати.

Овие фунгициди претставуваат одлична хемиска заштита од болеста сечење при двојна инфекција од двата патогени во тутунските леи.

**Клучни зборови:** сечење, фунгициди, ефикасност, Quadris 25 SC, Signum 33 WG, Orvego

## INTRODUCTION

The significance of tobacco seedlings for tobacco production is well-known. The success of tobacco yield and quality depends on a healthy and quality tobacco seedlings. But tobacco seedlings production can be degraded by the occurrence of many diseases. The most destructive is damping off disease. It can occur in all stages of development of seedlings, starting from the germination of seeds to the full growth.

The causing agents of the disease are *Rhizoctonia solani* and *Pythium debarianum*. In many cases, there is the mutual infection. Therefore, control of the disease is very hard.

The soil pathogen *Rhizoctonia solani* is difficult to control because it is common in various soil types and has many hosts. The fungus causes serious damage to many important field and horticultural crops (Nunez, 2005).

*Pythiace* family (to which it belongs *Pythium debarianum*) are considered opportunistic fungi preying on weakness and taking advantage of conditions which are not ideal for the plant. They can reproduce sexually (in the form of oospores), as well as asexually. They are almost always found in the root system (Morel Diffusion, 2017).

The symptoms they caused are similar. The true determination is possible only with microscopy and isolation of the pathogen. But, in practice this is unachievable.

The chemical control is still the most effective method of protection from the pathogens. But, its control is very difficult because of similarity of symptoms and wrong determination which lead to the noneffective control i.e use of non compatible fungicides.

The biggest problem is the dual infection which is very often, therefore the use of the true control is necessary. Targeted chemical control strategies become limited when more than one pathogenic agent contributes to the disease as the application of the specific substance may not necessarily result in succesful disease management (Lamichhane and Venturi, 2015).

There are numerous data on the activity of certain active substances, respective for each pathogen.

According to Koenning (2007), fungicides containing PCNB (Terrachlor), Iprodione (Rovral) or Azoxystrobin (Quadris) are effective in the control of *R. solani*. Recommended products for control of this pathogen in potatoes are fludioxonil, maneb, penthiopyrad, thiophanate-methyl, PCNB and azoxystrobin, with their trade names and modes of application (Schwartz and Gent, 2012). Azoxystrobin, trifloxystrobin, and tebuconazole are effective active ingredients against *Rhizoctonia* (Mocioni et al., 2003).

Azoxystrobin (Quadris) is recommended against root rot caused by *R. solani* (Bredehoert, 2012, Poindexter and Wenzel, 2013). For control of *R. solani* in soybean seedlings, the most recommended fungicides are strobilurins pyraclostrobin and trifloxystrobin (Mueller, 2014).

There are some reports for use strobilurins in tobacco. Azoxystrobin is used for control of *R. solani* in tobacco (Bertrand, 2012; LaMondia, 2012). Application of Quadris against this disease is also recommended by the manufacturer (Syngenta, 2006).

The most commonly used active ingredients in control of the disease is thiophanate-methyl. But, the fungicides Signum 33 WG and Quadris 25 SC achieved higher effectiveness in the control of pathogenic fungus *R. solani* in artificial inoculation and natural infection in seedbeds (Gveroska, 2015).

Prasad et al. (2014), testing in vitro ten fungicides against *Rhizoctonia solani* and *Pythium debarianum*, estimated no equal results for two pathogens. Tebuconazole +Trifloxystrobin, Propiconazole, Captan+Hexaconazole and Carbendazim showed 100 % inhibition in control of *Rhizoctonia solani*. In inhibitin of *Pythium debarianum*, Metalaxyl, Propiconazole, Tebuconazole +Trifloxystrobin, Metiram+Pyraclostrobin, Captan+Hexaconazole amd Thiram showed the best results.

Mihajlović et al. (2013) have tested 5 fungicides and 1 biofungicide against *P. aphanidermatum* – *in vitro* and *in vivo*. They pointed toxicity of Quadris in vitro and 100% efficacy of Fosetyl-Al in greenhouse assays.

As solution of *Pythium* control Chase (2013) lists etridiazole, mfenoxam, fluopicolide, phosphonates, strobilurins and *Trichoderma* sp. In tobacco seedling control propamocarb is the mostly used active ingredients against *P. debarianum*. BAYER (2019) also offers propamocarb hydrochloride (Banol®).

Meadows et al. (2017) reported different active ingredients against *Rhizoctonia* (benomyl, azoxystrobin, thiophanate-methyl, iprodione) and *Pythium* (etridiazole and metalaxyl) as well as broad spectrum fungicides in chemical control of damping off in seedlings. They suggested combined application in treatment of unknown pathogen or mutual infection.

Despite the great number of active ingredients and fungicides, control of these pathogenic fungi in our conditions was limited. Recently, many new fungicides and active ingredients have appeared on the market. But, there were not any results for the use in tobacco. This was one reason that imposed these investigations.

Effective control has to reduce the damages and achieve healthy seedlings by the minimum costs for plant protection products. It has to be performed using the contemporary standards of Sustainable Tobacco Production, which refers to the use of chemicals, too.

According to the previous practice for the investigated fungicides as well as the results from artificial inoculation, the aim of this study was determined - to find the most effective control of damping off in dual infection on tobacco seedlings in seedbeds.

## MATERIAL AND METHODS

Investigations were conducted in tobacco seedlings production in seedbeds, in Scientific Tobacco Institute-Prilep. The seedlings were cultivated in the usual way.

It was treated twice, in the stage before and the full development stage. Investigated fungicides –commercial names, active ingredients and used concentration are shown in Table 1.

They were applied in 3 replications of 10 m<sup>2</sup>, with the use of 1L water for 10m<sup>2</sup>.

They were chosen according to instructions, results from artificial inoculation and the previous practice.

Isolation and determination of the pathogen/s were made from the infected plants in check plots, before the treatments i.e. before the each estimation.

Two assessments of the intensity of damping off disease - the percentage of infected area were made, 10-15 days after the each treatment. Effectiveness of the tested fungicides for the each assessment was calculated by the formula of Abbott.

Figure 1 shows the average value (from the two estimations) of the fungicide's efficacy in trials.

**Table 1. Investigated fungicides**

Fungicide (Commercial name)	Active ingredient / s	Concentration (%)
Top-M 70%WP	70% thiophanate –methyl	0.1%
Proplant 722 SL	722g/l propamocarb	0.15%
Enervin WG	120 g/kg ametoctradin + 440 g/kg metiram	0.2%
Signum 33 WG	267 g/kg boscalid + 67g/kg pyraclostrobin	0.1%
Orvego™	300 g/l ametoctradin + 225 g/l dimetomorph	0.1%
Quadris 25 SC	250 g/l azoxystrobin	0.15%
Top-M 70%WP + Pronlant 722 SL	70% thiophanate –methyl + 722g/l propamocarb	0.1% + 0.15%
Signum 33 WG + Proplant 722 SL	267 g/kg boscalid + 67g/kg pyraclostrobin +	0.1% + 0.15%
Signum 33 WG + Orvego™	722g/l propamocarb 267 g/kg boscalid + 67g/kg pyraclostrobin +	0.1% + 0.1%
Orvego™ + Manfil 80WP	300 g/l ametoctradin + 225 g/l dimetomorph 300 g/l ametoctradin + 225 g/l dimetomorph +	0.1% +0.25%
	800 g/kg mancozeb	

## RESULTS AND DISSCUSION

Symptoms of the disease begins with the appearance of small watery spot at the base of the stalk which is starting to spread. The tissue becamec necrotic. So, the movement of water and nutrients is disabled, the plant falls on land as it is cuted - where the name comes from (Fig. 1a,b).

The tissue becomes disorganized, loses its firmness and take on a watery look. The young stalk is weakened and flops, bringing down the whole of the above-ground part of the plant. The disease may go so far as to make the plants literally dissolve and disappear from the surface, so it is also referred to as seedling melt (post-appartion melt) (Fig. 1c,d). Larger plants can get over thanks to their growth; the symptoms they exhibit are withering or yellowing of a leaf or a few leaves (Morel Diffusion, 2017).

The infection quickly spreads, infected fireplaces in its beds expand and converge, because much of the seedlings were destroyed (Fig 2 a,b,c). The danger is evident not only in seedlings production, but also in the whole tobacco production.

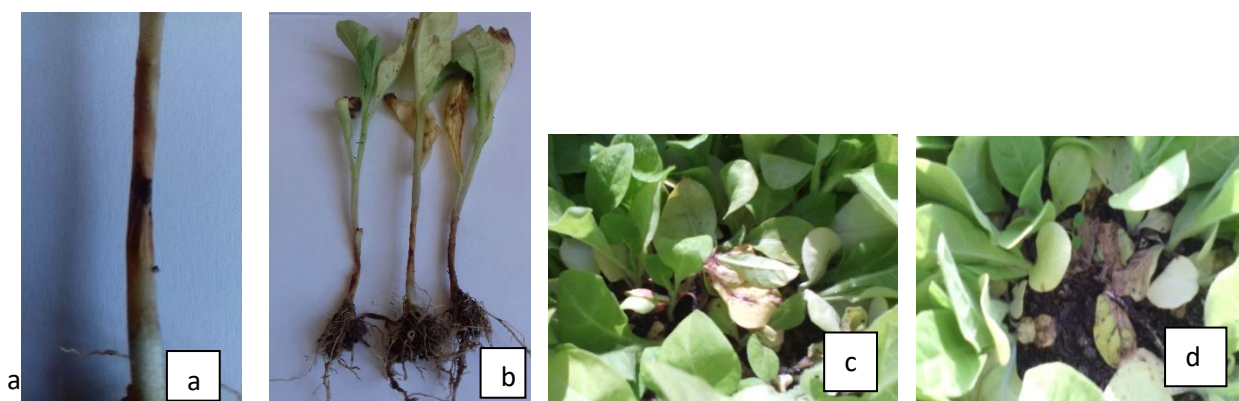


Figure 1 (a-d). Symptoms of the disease



Figure 2 (a-c). Expansion and merge of the infected sites by spreading the disease

The each test of the causing agent/s during investigations showed the presence of both pathogens *R. solani* and *P. debarianum*. Pure cultures of these fungi are presented in Figure 3 and 4.

They have different taxonomy i.e morphological, physiological, generic and reproductive characteristics (Ivanović, 1994). Both pathogenic fungi are well known damping off agents causing significant damage in nurseries (Blancard, 2012).

Symptoms they caused are similar and hardly to differentiating and their presence at the same time suggests that it is a double infection. According to Lamichhane and Venturi (2015) there are synergism between microbial pathogens in plant disease complex. The universality of synergism need to better consider in the future that one pathogenic agent can team up with others rather than acting alone. Therefore, that might have important implications in plant disease epidemiology and consequent development of effective disease control strategies.

These efforts for successful damping off disease management in tobacco via these assays yielded the following results.

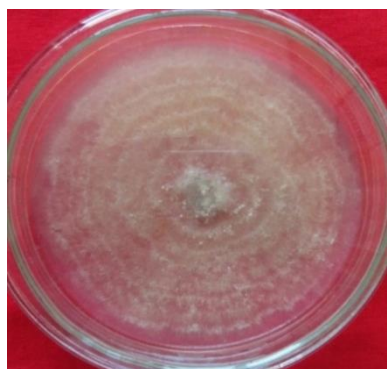


Figure 3. *Rhizoctonia solani* –pure culture



Figure 4. *Pythium debarianum* – pure culture

The mean value (from individual replications) of disease intensity ranged from 0.06% in Proplant 722 SL to 0.19% in contact fungicide Enervin WG. The highest intensity was observed in the control, whereas in the seedlings treated with Quadris 25 SC no disease was present (Table 2).

According to intensity, the tested fungicides showed adequate efficacy. The lowest effectiveness was shown by the Enervin WG and the highest by Quadris 25 SC (100%).

In this rating Proplant 722 SL showed good efficiency – 82.35%. In contact fungicide Enervin WG it is only 44.12%. Other preparations and combinations showed about 70% efficacy, with the highest value in Orvego TM and its combination - Orvego TM + Manfil 80WP (79.41%).

Most of fungicides showed lower values at the second estimation. It ranges from 0.01% in Orvego TM + Manfil 80WP and Signum 33 EG + OrvegoTM to 0.26% in Enervin WG. However, treatment with Top-M 70% WP as well as the Signum 33 EG + Proplant 722 SL also showed a very low attack intensity – 0.02%. As the previous estimation, at Quadris 25 SC had no disease.

Calculated effectiveness has higher values than in the first estimation. It is the smallest in the Enervin WG - almost the same as in the first rating. Very high efficiency is achieved with Orvego TM + Manfil 80WP and Signum 33 EG + OrvegoTM – 97.87% as well as Top-M 70% WP and Signum 33 EG + Proplant 722 SL-95.74%. The effectiveness of Signum EG is also excellent -93.62%. Orvego TM has high value, too -82.98%. Application of Proplant 722 SL alone or in combination with Top-M had a lower efficacy -72.4% and 78.73%.

**Table 2. Intensity of disease in the first estimation and effectiveness of fungicides**

Variant (fungicide) Concentration (%)	Intensity			Average value	Effectiveness
	Replication				
	I	II	III		
Check Ø	0.36	0.42	0.24	0.34	-
Top-M 70%WP 0.1%	0.08	0.08	1.15	0.10	70.59
Proplant 722 SL 0.15%	0.09	0.07	0.02	0.06	<b>82.35</b>
Enervin WG 0.2%	0.23	0.13	0.21	0.19	44.12
Signum 33 WG 0.1%	0.04	0.11	0.09	0.08	76.47
Orvego <sup>TM</sup> 0.1%	0.04	0.09	0.08	0.07	79.41
Quadris 25 SC 0.15%	0	0	0	0	<b>100.00</b>
Top-M 70%WP +Proplant 722 SL 0.1% + 0.15%	0.11	0.04	0.09	0.08	76.47
Signum 33 WG + Proplant 722 SL 0.1% + 0.5%	0.08	0.12	0.1	0.10	70.59
Signum 33 WG +Orvego <sup>TM</sup> 0.1% + 0.1%	0.10	0.1	0.09	0.10	70.59
Orvego <sup>TM</sup> +Manfil 80WP 0.1% +0.25%	0	0.13	0.07	0.07	79.41

The average value of the fungicide effectiveness is presented in Graph. 1. The effect of fungicide's treatment is shown in Figure 5-7.

The least effective was contact fungicide Enervin WG, which is quite understandable. The standard Top-M 70% WP + Proplant 722 SL combination also had poor results. Quadris 25 SC-100% achieved the best effectiveness during the tests. But also Signum 33 EG with 85.05%, as well as its combinations - Signum 33 EG + Orvego<sup>TM</sup> (84.23%) and Signum 33 EG + Proplant 722 SL (83.17%).

The high effectiveness (88.64%) was achieved with treatment with Orvego<sup>TM</sup> + Manfil 80WP.

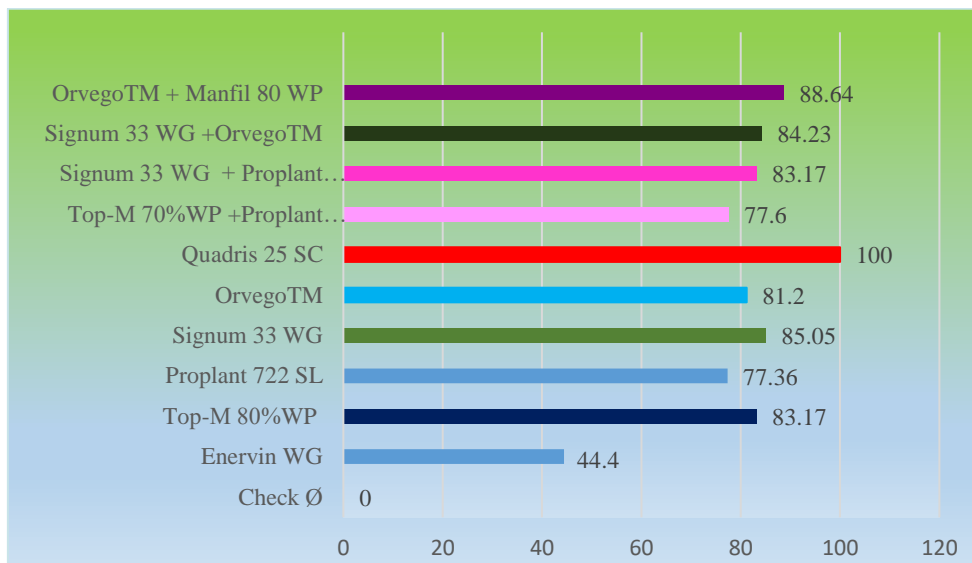
**Table 3. Intensity of disease in the second estimation and effectiveness of fungicides**

Variant (fungicide) Concentration (%)	Intensity			Average value	Effectiveness
	Replication				
	I	II	III		
Check Ø	0.48	0.43	0.50	0.47	-
Top-M 70%WP 0.1%	0	0	0.07	0.02	<b>95.74</b>
Proplant 722 SL 0.15%	0.16	0.11	0.12	0.13	72.34
Enervin WG 0.2%	0.29	0.33	0.16	0.26	44.68
Signum 33 WG 0.1%	0.05	0	0.04	0.03	<b>93.62</b>
Orvego <sup>TM</sup> 0.1%	0.12	0.13	0	0.08	82.98
Quadris 25 SC 0.15%	0	0	0	0	<b>100.00</b>
Top-M 70%WP +Proplant 722 SL 0.1% + 0.15%	0.11	0.12	0.08	0.10	78.73
Signum 33 WG + Proplant 722 SL 0.1% + 0.15%	0.01	0.06	0	0.02	<b>95.74</b>
Signum 33 WG +Orvego <sup>TM</sup> 0.1% + 0.1%	0	0.04	0	0.01	<b>97.87</b>
Orvego <sup>TM</sup> +Manfil 80WP 0.1% +0.25%	0.04	0	0	0.01	<b>97.87</b>

Achieved effectiveness of strobilurins in these assays confirmed the findings of the others: (Bertrand (2012), LaMondia (2012), Syngenta (2006). Towards effective protection of many crops with Azoxystrobin against root diseases (Bredehoert, 2012; Poindexter and Wenzel, 2013; Schwartz and Gent, 2012; Koenning (2007) tobacco is also joining. This active ingredient showed the best effectiveness in the case of dual infection in the seedbeds. The efficacy of Quadris is due to the direct effect of azoxystrobin on the inoculum in the soil, as well as its effective combination with plant tissue (LaMondia, 2012).

According to BASF (2008), combination of pyraclostrobin and boscalid (Signum fungicide) - has preventive and systemic activity for use against certain diseases in various crops. This includes tobacco / tobacco seedling because this combination has shown high efficacy in control of the damping off disease. The efficacy of Signum may be due to the fact that the two active ingredients are an excellent combination of two different biochemical modes of action on cellular respiration of fungi (Hauke et al., 2004).





Graph 1. Effectiveness of the investigated fungicides (average value of two estimations)



Figure 5. Seedlings treated with Quadris 25

SC



Figure 6. Seedbed - Proplant 722 SL + Top M 70% WP



Figure 7. Seedbeds - Quadris 25 SC (left) and Orvego<sup>TM</sup> +Manfil (right)

Combination of many active ingredients with those of Signum with different mode of action achieved a big success, too. It has to be concluded that mixtures combine the effectiveness of different fungicides for broad-spectrum activity.

If the specific fungus causing damping-off is not known. Meadows et al (2017) suggested the broad spectrum fungicide or two specific fungicides (etridiazole + thiophanate methyl) either combination of fungicides (benomyl plus. etridiazole or metalaxyl). More precisely, each a.i. in combination should be acts against the specific pathogen in mutual control of *Rhizoctonia* and *Pythium*.

These statements are confirmed by these investigations, too. Combinations of fungicides (active ingredients), combination of a.i. with preventive and systemic action and use of fungicides with a wide range of activities had the best results in control of damping off in dual infection by two pathogens.

### CONCLUSIONS

- Damping off disease in tobacco seedlings production is a serious problem especially in the dual infection by two pathogens.
- This model of fungicide treatment offers a new opportunity for effective control disease.
- The most effective control is a fungicide who acts against two pathogens or combination who act separately to each pathogen or simultaneously.
- Fungicide Quadris 25 SC (250g/l azoxystrobin) 0.1% had the best effectiveness - 100%
- The highest effectiveness is achieved by two combinations: Orvego (300g/l ametoctradin + 225g/l dimetomorph) 0.1% + Manfil 80WP (800 g/kg mancozeb) 0.25% - 88.64% and Orvego 0.1% + Signum 33 WG 0.1% - 84.23% effectiveness.
- The common fungicides used even in their combination had a weaker results than new active ingredients.
- The pointed fungicides and combinations provide the excellent chemical control for damping off disease in dual infection by two pathogenic fungi in tobacco beds.
- These results contribute to enlarging the list of active ingredients for tobacco seedling protection.

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