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INFLUENCE OF SOME FACTORS IN VITRO ON THE DEVELOPMENT OF *DIAPORTHE INCARCERATA* (BERK. & BROOME) NITSCHKE

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Abstract: Diaporthe incarcerata, known as pathogen causing the dieback of Rosa branches was detected in many orchards in Romania. Our investigations approached a series of bio-ecological aspects of this pathogen: isolation, purification and obtaining of the pathogen; determination and identification of pathogen; establishing in vitro parameters of fungal development (carbon source). The isolate used in this study was obtained from Rosa spp. branched and was cultivated on three culture media: potato dextrose agar (PDA), malt extract agar and water agar which included five saccharides: glucose, fructose, arabinose, cellulose and starch. When the pathogen was grown on PDA, the most favourable carbon sources for the development were arabinose, glucose and starch. In case of extract agar, the most favourable carbon source were fructose and arabinose, followed by glucose, starch and cellulose. When the fungus was growth on water agar, the sporulation was completely inhibited.

Keywords: *Diaporthe*, in vitro, carbon source

Introduction

Diaporthe (anamorph *Phomopsis*) is a wide genus of fungi [5]. Over 800 species of *Phomopsis* were described on the plant host basis [8]. Until recently, it was assumed that species of *Phomopsis* were restricted to specific plant hosts at the species or, at the least, genus level. Recent studies by Farr [4], Rehner and Uecker [6], suggest that delimiting species within the genus *Phomopsis* is more complex than had been previously recognized.

In Romania there were described 87 taxons on 130 species of host plants which have great importance in economy and flora [2].

Phomopsis incarcerata (Sacc.) Höhn., conidial state of *Diaporthe incarcerata* (**Diaporthales, Diaporthaceae**) is known as the principal pathogen which can cause the dieback of the branches and twigs of *Rosa* spp. [1, 3]. It was isolated and purified on the potato dextrose agar (PDA). Later on we established the influences of the culture media of the fungal development.

The research concerning the bio-ecological aspects of this pathogen continued with including in the experimental protocol of another parameters of the in vitro development such as the carbon sources.

Materials and Methods

The isolates of PR2 used in this study were obtained from branches of *Rosa* spp. The isolates were cultivated on three culture media, namely: potato dextrose agar (PDA), malt extract agar and water agar. In these culture media were included five saccharides, tested as the carbon sources: glucose, fructose, arabinose, starch and cellulose. The experiments included six variants for each medium and minimum four repetitions per variant.

The results obtained were statistically analysed with SPSS 10 programme.

Results

On the potato dextrose agar (PDA) which included the five saccharides, 3 days after the culture initiation, there were obtained the most favourable carbon sources for the development of

the *Phomopsis incarcerata*. They were: glucose (4,425 cm), arabinose (4,325 cm) and starch (4,025 cm). Less favourable were fructose (3,750 cm) and cellulose (3,450 cm). After 7 days, the saccharides which stimulated the growth were arabinose (6,700 cm), starch (6,500 cm) and glucose (6,300 cm). After 10 days, an uniform growth was observed in case of all saccharides, while the isolates had the colony diameter between 6,850 and 7,000 cm

Regarding the sporulation of the pathogen, the first pycnidia appeared 10 days after inoculation in the Petri dishes which contained glucose, fructose, arabinose and cellulose; in case of starch variant, the pycnidial buttons were formed after 21 days (Fig. 1).

The statistical survey of data is presented in table 1.

On the malt extract agar which included the five saccharides, 3 days after inoculation, the most favourable carbon sources for the development of fungus were starch (2,150 cm), glucose (2,075 cm) and arabinose (2,000 cm). After 7 days, the saccharides which stimulated growth were fructose (5,825 cm) and arabinose (5,800 cm), followed by glucose (4,475 cm), starch (4,30 cm) and cellulose (3,40 cm).

Concerning the sporulation of the pathogen, the first pycnidia appeared after 10 days of the inoculation in the Petri dishes which contained fructose; in glucose, arabinose and starch variants, the pycnidial buttons formed after 14 days, in cellulose variant the first pycnidia appeared after 21 days.

Table 1: Statistic analysis of data obtained *in vitro* on PDA after 7 days

Variant (I)	Variant (J)	Mean Difference (I-J)	Std. Error	95 % confidence interval	
				Lower Bound	Upper Bound
1	2 - fructose	0,4250	0,2668	-0,1355	0,9855
	3- arabinose	-0,4000	0,2668	-0,9605	0,1605
	4 - control	1,0250*	0,2668	0,4645	1,5855
	5 - starch	-0,2000	0,2668	-0,7605	0,3605
	6 - cellulose	0,8250*	0,2668	0,2645	1,3855
2	1- glucose	-0,4250	0,2668	-0,9855	0,1355
	3- arabinose	-0,8250*	0,2668	-1,3855	-0,2645
	4 - control	0,6000*	0,2668	$3{,}948_{E}$-02	1,1605
	5 - starch	-0,6250*	0,2668	-1,1855	$-6{,}4481_{E}$-02
	6 - cellulose	0,4000	0,2668	-0,1605	0,9605
3	1 - glucose	0,4000	0,2668	-0,1605	0,9605
	2 - fructose	0,8250*	0,2668	0,2645	1,3855
	4 - control	1,4250*	0,2668	0,8645	1,9855
	5 - starch	0,2000	0,2668	-0,3605	0,7605
	6 - cellulose	1,2250*	0,2668	0,6645	1,7855
4	1 - glucose	-1,0250*	0,2668	-1,5855	-0,4645
	2 - fructose	-0,6000*	0,2668	-1,1605	$-3{,}9481_{E}$-02
	3 - arabinose	-1,4250*	0,2668	-1,9855	-0,8645
	5 - starch	-1,2250*	0,2668	-1,7855	-0,6645
	6 - cellulose	-0,2000	0,2668	-0,3605	0,3605
5	1 - glucose	0,2000	0,2668	-0,3605	0,7605
	2 - fructose	0,6250*	0,2668	$6{,}448_{E}$-02	1,1855
	3 - arabinose	-0,2000	0,2668	-0,7605	0,3605
	4 - control	1,2250*	0,2668	0,6645	1,7855
	6 - cellulose	1,0250*	0,2668	0,4645	1,5855
6	1 - glucose	-0,8250*	0,2668	-1,3855	-0,2645
	2 - fructose	-0,4000	0,2668	-0,9605	0,1605
	3 - arabinose	-1,2250*	0,2668	-1,7855	-0,6645
	4 - control	0,2000	0,2668	-0,3605	0,7605
	5 - starch	-1,0250*	0,2668	-1,5855	-0,4645

* the mean difference is significant at the 0,05 level

The statistical survey of data is presented in table 2.

On the water agar which included the five saccharides, 3 days after inoculation, the pathogen was not growth. After 7 days, the saccharides which stimulated growth were fructose (4,750 cm), glucose (4,475 cm) and starch (4,325 cm), and after another two weeks, the favourable carbon source were fructose (5,175 cm), starch (5,100 cm) and glucose (4,950 cm). The first pycnidial buttons were observed after 21 days in all variant, except the control.

If we compare the influence of the carbon sources included into different culture media (PDA, malt extract agar, water agar), we will reveal the differences between the rate of growth as well as the gross morphology of pycnidia. When the pathogen was grown on PDA, the mycelium growth was greater, the pycnidia were numerous and the sporulation was strong. When the fungus was grown on water agar with all five studied saccharides, the mycelium, the produced pycnidia had small diameters <0.5 mm and the sporulation was completely inhibited.

Our results are comparable with those obtained by Ulea E. at the fungus *Phomopsis viticola* and by Tatiana Şesan at the *Phomopsis occulta* known weak parasite in conifers [7, 9].

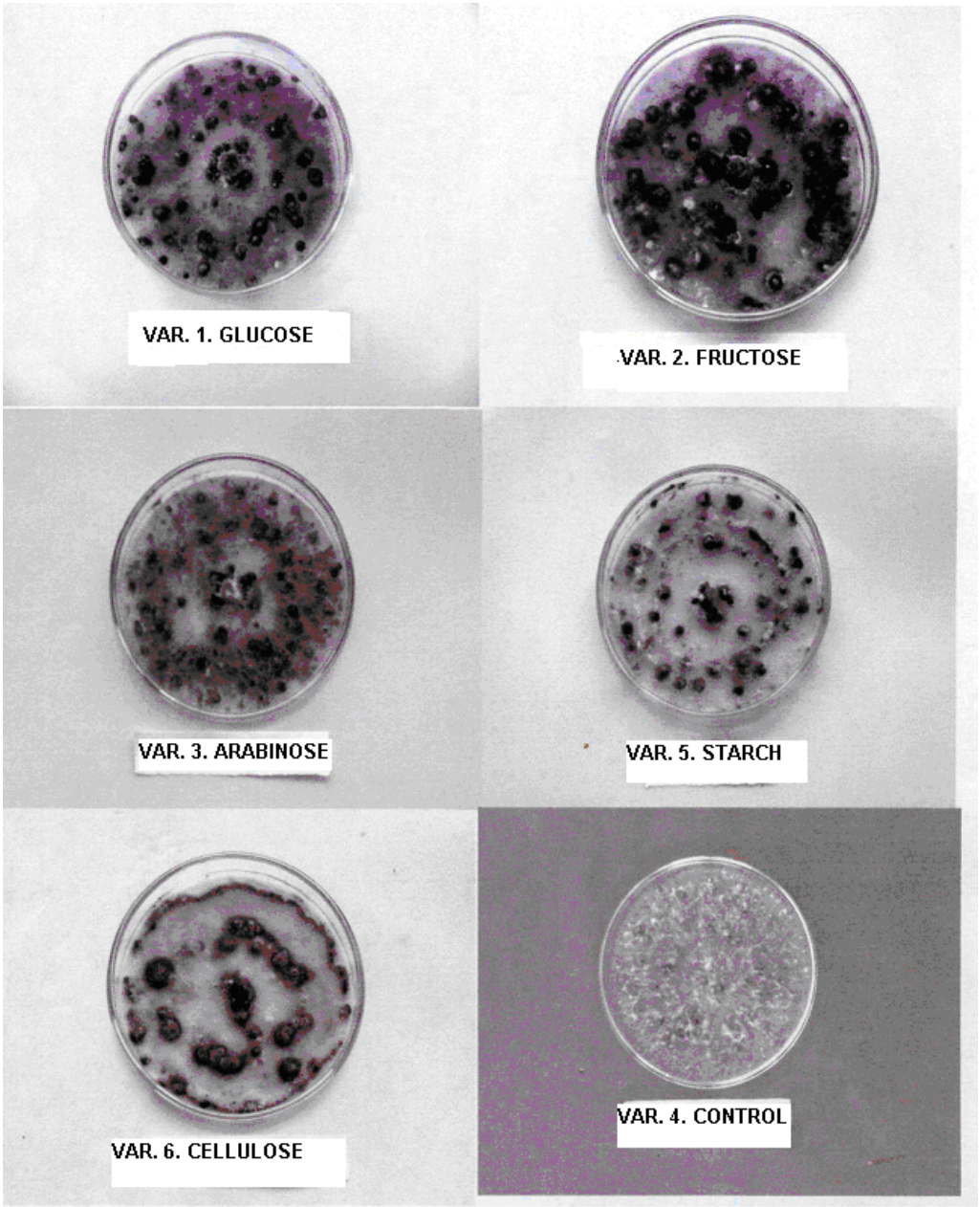

Fig. 1: The growth and development of the *Diaporthe incarcerata* on PDA medium with different saccharides. (var. = variant)

Table 2: Statistic analysis of data obtained *in vitro* on malt extract agar after 7 days

Variant (I)	Variant (J)	Mean Difference (I-J)	Std. Error	95 % confidence interval	
				Lower Bound	Upper Bound
1	2 - fructose	- 1,3500*	0,3652	- 2,1173	-0,5827
	3 - arabinose	- 1,3250*	0,3652	- 2, 0923	-0,5577
	4 - control	0,6500	0,3652	-0,1173	1,4173
	5 - starch	0,1750	0,3652	-0,5923	0,9423
	6 - cellulose	1,0750*	0,3652	0,3077	1,8423
2	1- glucose	1,3500*	0,3652	0,5827	2,1173
	3 - arabinose	$2{,}500_{E}$-02	0,3652	-0,7423	0,7923
	4 - control	2,0000*	0,3652	1,2327	2,7673
	5 - starch	1,5250*	0,3652	0,7577	2,2923
	6 - cellulose	2,4250*	0,3652	1,6577	3,1923
3	1 - glucose	1,3250*	0,3652	0,5577	2,0923
	2 - fructose	$-2{,}5000_{E}$-02	0,3652	-0,7923	0,7423
	4 - control	1,9750*	0,3652	1,2077	2,7423
	5 - starch	1,5000*	0,3652	0,7327	2,2673
	6 - cellulose	2,4000*	0,3652	1,6327	3,1673
4	1 - glucose	-0,6500	0,3652	-1,4173	0,1173
	2 - fructose	-2,0000*	0,3652	-2,7673	-1,2327
	3 - arabinose	-1,9750*	0,3652	-2,7423	-1,2077
	5 - starch	-0,4750	0,3652	-1,2423	0,2923
	6 - cellulose	0,4250	0,3652	-0,3423	1,1923
5	1 - glucose	-0,1750	0,3652	-0,9423	0,5923
	2 - fructose	-1,5250*	0,3652	-2,2923	-0,7577
	3 - arabinose	-1,5000*	0,3652	-2,2673	-0,7327
	4 - control	0,4750	0,3652	-0,2923	1,2423
	6 - cellulose	0,9000*	0,3652	0,1327	1,6673
6	1 - glucose	-1,0750*	0,3652	-1,8423	-0,3077
	2 - fructose	-2,4250*	0,3652	-3,1923	-1,6577
	3 - arabinose	-2,4000*	0,3652	-3,1673	-1,6327
	4 - control	-0,4250	0,3652	-1,1923	0,3423
	5 - starch	-0,9000*	0,3652	-1,6673	-0,1327

* the mean difference is significant at the 0,05 level

Conclusions

1. *Phomopsis incarcerata* (Sacc.) Höhn., conidial state of *Diaporthe incarcerata* is known as the principal pathogen which can cause the dieback of the branches and twigs of *Rosa* spp.
2. On the potato dextrose agar (PDA) the saccharides which stimulated growth were arabinose, starch and glucose and first pycnidia appeared 10 days after inoculation in Petri dishes which contained glucose, fructose, arabinose and cellulose.
3. On the malt extract agar the saccharides which stimulated growth were fructose and arabinose, followed glucose, starch and cellulose and the first pycnidia appeared 10 days after inoculation in Petri dishes which contained fructose.
4. On the water agar the mycelium was hardly visible in Petri dishes and the sporulation was completely inhibited.

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INFLUENŢA UNOR FACTORI IN VITRO ASUPRA CREŞTERII ŞI DEZVOLTĂRII PATOGENULUI *DIAPORTHE INCARCERATA* (BERK. & BROOME) NITSCHKE

(Rezumat)

Diaporthe incarcerata (anamorfa *Phomopsis incarcerata*) este un patogen care cauzează cancerul ramurilor de *Rosa* spp., fiind semnalat în multe grădini din România. Investigaţiile noastre au urmărit o serie de aspecte bio-ecologice: izolarea, purificarea şi determinarea patogenului; influenţa unor parametri (diferite surse de carbon) *in vitro* asupra creşterii şi dezvoltării patogenului. Izolatele din acest studiu au fost obţinute de pe lăstari de *Rosa* spp. pe mediu CGA (cartof-glucoză-agar). Ulterior ele au fost cultivate pe trei medii de cultură (CGA, malţ agar şi apă agarizată) îmbogăţite cu 5 zaharide: glucoză, fructoză, arabinoză, celuloză şi amidon. Când patogenul a fost crescut pe mediul CGA care conţinea cele 5 zaharide luate în studiu, miceliul a crescut rapid în variantele cu arabinoză, glucoză şi amidon. Pe malţ agar, cele mai favorabile zaharide creşterii ciupercii au fost fructoza şi arabinoza, iar pe apă agarizată ciuperca a crescut, dar sporularea ciupercii a fost inhibată.