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INTERACTIONS OF RECOMBINED MYCELIA OF THE FUNGUS FOMITOPSIS PINICOLA (SOW. EX FR.) KARST. ON PDA MEDIUM

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The interaction of cultures from the recombined mycelia of the fungus *Fomitopsis pinicola* (Sow. ex Fr.) Karst. was studied on PDA medium. The cultures were isolated from different carpophores and from different segments of the same carpophore originating from a felled beech tree. The interactions show that recombined mycelia of this species can be divided into numerous incompatible physiological “races” or “pathotypes” distinguished also by morphological features of the mycelia.

Key words: *Fomitopsis pinicola*, recombined mycelia, zone of interaction, variety line, pathotype

INTRODUCTION

One of the most dynamic and significant aspects of biology is that the characters of individuals within a species are not "permanent", or unchangeable, i.e. they are not identical and all individuals result from a sexual reproduction. This phenomenon also occurs in fungi, i.e. the individuals originate from the reproductive organs of sexual origin (oospores, ascospores, basidiospores), in parasitic angiosperms (seed), nematodes (fertile eggs) and plants (seed). Even bacteria have the mechanisms for carrying the genetic information.

The analysis of a beech log lying in the forest for a long time shows the presence of numerous carpophores of the epixylous fungus *Fomitopsis pinicola*

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throughout the log. After the isolation of fungus from these carpophores, but also from different segments of the same carpophore, morphological differences were recorded between the obtained recombined mycelia (in Petri dish, mycelia develop by the fusion of hyphae originating from different basidiospores). The above data initiated the study of the interaction between these potential "races" or "pathotypes" of the fungus *Fomitopsis pinicola* or other fungi (e.g. *Phellinus igniarius* /VERRALL, 1937/, *Fomes cajanderi* /ADAMS and ROTH, 1967/, *Polyporus schweinitzii* /BARET, USČUPLIĆ, 1971/, *Fomes pini* /USČUPLIĆ, LAZAREV, unpublished/.

The aim of this paper is to assess the behaviour of recombined mycelia of the fungus *Fomitopsis pinicola* (potential "variety" lines, "physiological races" or "pathotypes") based on their morphological-anatomic characters.

MATERIAL AND METHODS

Twenty-three sporophores (Fig. 1) were taken from the beech log in the virgin forest Peručica (Bosnia and Herzegovina), on which numerous carpophores of the fungus *Fomitopsis pinicola* formed. In the laboratory, in the aseptic chamber and under standard mycological and phyto-pathological techniques, the fragments

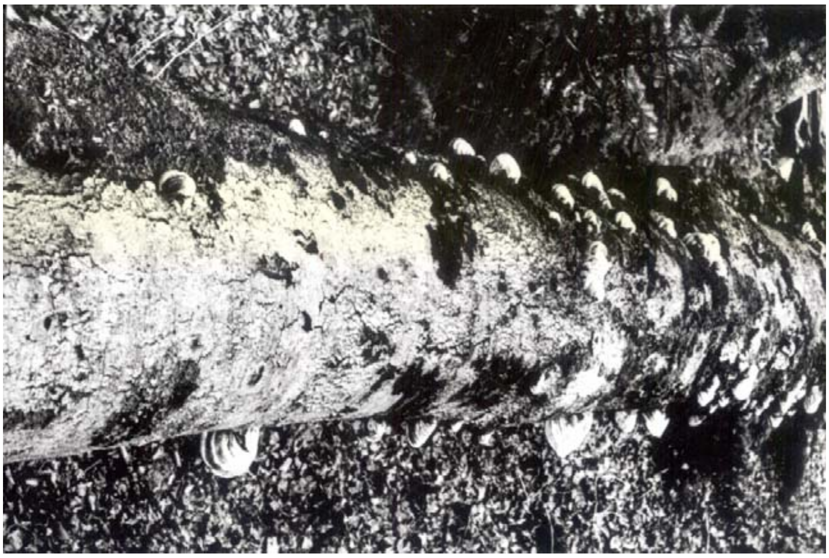

Fig. 1. Felled beech log covered by carpophores of the fungus *Fomitopsis pinicola*

of the hymenal layer (0.5 x 0.5 cm) representing the inoculum were taken and placed in Petri dishes on PDA (potato dextrose agar). The inocula from 22 carpophores were numbered from 1 to 22. From the carpophore number 23, inocula were taken from nine different segments of the hymenium layer and marked in alphabetic order from 23a to 23i. After the reisolation, pure cultures of recombined mycelia were obtained from each individual carpophore, but also from one carpophore. They differed in morphology and growth vigour under the same conditions of room temperature (18-22^0 C). The fragments of different cultures of mycelia from different carpophores (1-22) and from the same carpophore (23a-23i) were sown in pairs in all combinations, After their growth, the behaviour of parallel sown colonies was monitored. We analysed the morphological similarity (based on mycelium density, vigour and colour nuance) and aversion (coloured and discoloured zone of aversion, absence of aversion).

RESULTS AND DISCUSSION

The results of interaction of 465 combinations of parallel sown recombined mycelia of the fungus *Fomitopsis pinicola* on PDA are presented in the Tables 1 (isolates originate from different carpophores) and 2 (isolates originate from the same carpophore) and collectively in the Histogram 1. The results presented in the Tables and in the Histogram show as follows:

- 156 pairs form the dark zone of aversion, although the pairs are morphologically similar;
- 76 pairs form the discoloured aversion line, although the pairs are morphologically similar;
- mycelia of 153 pairs are morphologically different, and they form the dark aversion line (Fig. 2);

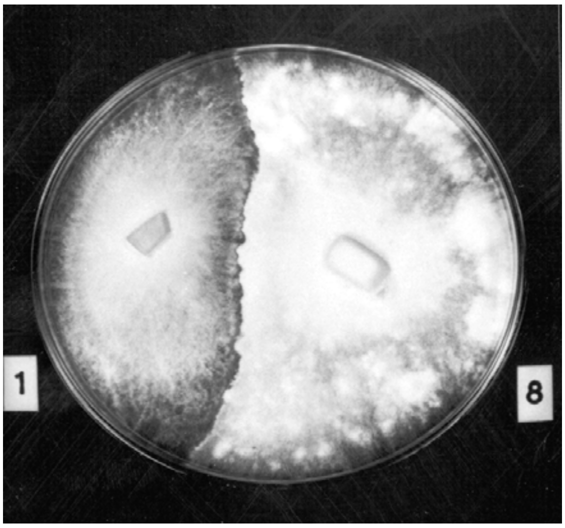

Fig. 2. Interaction of *F. pinicola* isolates from different fruiting bodies on the same tree. It is distinguished by the dark aversion line in the contact zone of mycelia. Morphological differences between mycelia are significant

- mycelia of 51 pairs are morphologically different and with a discoloured aversion line;

- in 29 pairs, mycelia are morphologically very similar and between them there is no aversion (Fig. 3).

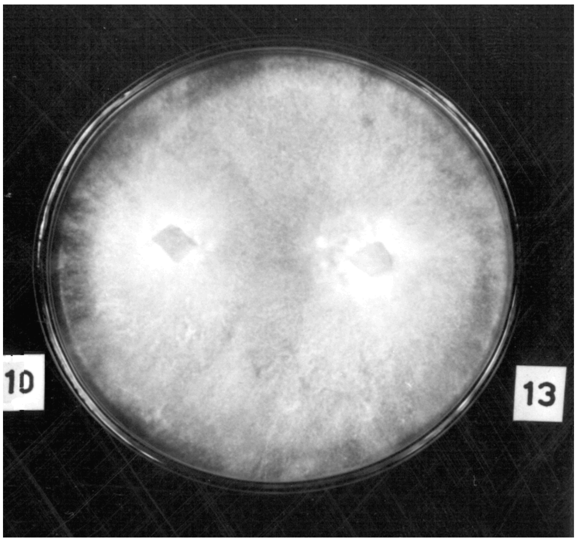

Fig. 3. Interaction of *F. pinicola* isolates from different carpophores on the same tree, distinguished by the absence of aversion. Morphological differences between mycelia are not visible

The aversion was absent between the following isolates:

isolate 1= 7;
isolate 3= 19;
isolate 4= 12 = 23a = 23b;
isolate 5= 8;
isolate 9= 14 = 17;
isolate 10= 13;
isolate 12= 23a = 23b;
isolate 14= 17;
isolate 18= 21;
isolate 23a= 23b;
isolate 23c= 23e = 23f = 23g = 23h = 23i;
isolate 23e= 23f = 23g = 23h = 23i;
isolate 23f= 23g = 23h = 23i;
isolate 23g= 23h = 23i;
isolate 23h= 23i

In the above 29 pairs, the mycelia are morphologically similar, compatible and coiled, so that after one-month development on PDA, one cannot differentiate the two isolates. This phenomenon is especially present in the pairs which do not originate from the same sporophore, probably because of the lower percentage of heterogeneous hyphae in one fruiting body.

However, in 436 pairs the interaction of synthetic heterokaryons shows that they originate from the pairs of heterogeneous basidiospores which are the base of the potential interaction of the obtained isolates. A number of paired myce-lia cultures (309) form a dark brown aversion zone when they originate from different sources, confronting on the same medium. A similar confrontation, without the dark zone of interaction was observed in 127 combinations.

Table 1. Interactions of recombined F.pinicola *mycelia from different carpophores*

	1	2	3	4	5	6	7	8	9	10	11
1	=	xx +	xx +	xx -	xx -	xx -	0	xx -	xx -	xx +	xx -
2		=	xx +	xx +	xx +	xx -	xx -	xx -	xx +	xx +	x -
3			=	xx +	xx -	xx -	xx +	xx -	xx +	xx +	xx -
4				=	xx -	x -	x -	xx -	xx -	xx +	x -
5					=	xx -	xx -	0	xx -	xx -	xx -
6						=	xx -	xx -	xx -	xx -	x -
7							=	xx +	xx +	xx +	xx +
8								=	xx +	x +	xx -
9									=	xx -	xx -
10										=	xx +
11											=
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											

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	12	13	14	15	16	17	18	19	20	21	22
1	x +	xx +	xx +	xx +	xx +	xx +	xx +	xx +	xx -	xx +	x +
2	xx +	xx +	xx +	xx +	x +	xx +	xx -	xx +	xx -	xx +	xx +
3	xx +	xx +	xx +	xx +	xx +	xx +	xx -	0	xx -	xx -	xx -
4	0	xx -	x +	xx +	x +	x -	x -	xx +	xx -	x -	x -
5	xx -	xx +	xx -	xx -	xx -	xx -	xx -	xx -	xx -	xx -	xx -
6	x -	xx -	xx -	xx -	x -	xx -	xx -	xx -	xx -	xx -	x -
7	x +	xx +	xx +	xx +	xx +	xx +	xx +	xx +	xx -	xx +	xx +
8	x -	xx +	xx -	xx +	xx +	xx +	xx +	xx -	xx -	xx -	xx -
9	xx +	xx -	0	xx +	xx +	0	xx -	xx +	xx -	xx -	x -
10	xx +	0	xx -	xx +	xx +	xx +	xx -	xx +	xx -	xx -	x -
11	x +	x -	xx +	xx -	x -	xx -	x -	x -	xx -	x -	x -
12	=	xx +	xx +	xx -	x +	xx +	xx +	xx -	xx +	x +	x -
13		=	xx +	xx +	xx +	xx +	xx -	xx +	xx -	xx +	xx +
14			=	xx +	x +	0	xx +	xx +	xx -	xx -	x +
15				=	xx -	xx +	xx +	xx +	xx -	x -	x +
16					=	xx +	x +	xx +	xx -	x +	x +
17						=	xx -	xx -	xx -	x +	xx +
18							=	xx -	xx -	0	x +
19								=	xx -	xx -	xx +
20									=	xx -	x -
21										=	x +
22											=

xx = dark aversion line; x = discoloured aversion line; 0 = absent aversion; + = mycelia; morphologically identical; = mycelia morphologically different

The interactions in the contact zone of different types ("races") of *F. pinicola* were monitored by the microscope. The contact of two types of mycelia results in the fusion of the first hyphae. Discoloration (the change of colour into dark aversion line) starts when fine "knots" form along the zone of initial diversion. Further growth of hyphae in this zone is slowed down, but airy hyphae bend on one side or both sides in the contact zone. Darker staining is observed in hyphae with fewer septa. In the formed zone of interaction, there are undamaged hyphae and hyphae in different degrees of cytoplasmatic decomposition. It seems that the decomposed hypha cells lead to the change of colour in the zone of interaction. So it can be inferred that the zone of interaction results from the decomposition of hyphae in the space of initial fusion of mycelia. The differences in the density and width of the zone of interaction reflect the degree of aversion of two mycelia sown one against the other. In some combinations, there is only a partial interaction (inhibition) of paired colonies.

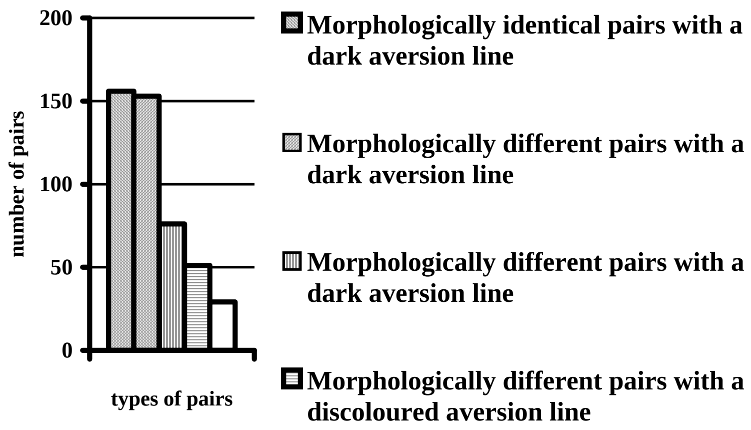
Table 2. Interactions of recombined Fomitopsis pinicola *mycelia from the same carpophore*

	23a	23b	23c	23d	23e	23f	23g	23h	23i
23a	=	0	x	xx	x	x	x	x	x
			-	+	-	+	+	+	+
23b		=	x	xx	x	xx	x	x	x
			+	+	-	-	-	+	+
23c			=	x	0	0	0	0	0
				+					
23d				=	x	x	x	x	x
					+	+	+	+	+
23e					=	0	0	0	0
23f						=	0	0	0
23g							=	0	0
23h								=	0
23i									=

xx = dark aversion line; x = discoloured aversion line; 0 = absent aversion; + = mycelia;
morphologically identical; = mycelia morphologically different

In fungi, there are two general mechanisms of variability: recombination and mutation.

The recombination of nuclei in fungi occurs primarily during sexual reproduction when two haploid (1n) nuclei contain a slightly different genetic material, and together they form a zygote. The zygote, sooner or later, divides in meiosis forming new haploid cells (gametes, spores, mycelia). The recombination of genetic characteristics occurs during meiosis as the result of crossing when the parts of chromatids (and genes on them) of one chromosome pair exchange with the parts of chromatids of the other chromosome pair. The recombination of genes of two parental nuclei can occur also in the zygote, but also during the mitotic division of cells during individual growth resulting in significant genetic changes. In fungi, the haploid nucleus or gametes often divide mitotically making the haploid mycelium and spores, which results in genetically different groups of relatively homogeneous individuals which can create wide asexual populations till the following sexual cycle (crossing over). In most fungi with sexual reproduction, population variability occurs primarily through the segregation and recombination of genes during the process of meiosis of zygotes.



Histogram 1: Results of interaction of parallel sown recombined mycelia of *Fomitopsis pinicola*

Mutation is a more or less sudden change of the genetic material which carries an inherited change to the offspring. Mutations are expressed by the changes in the sequence of DNA bases, then by substitution of one base by another, or by addition or loss if one or more base pairs. Additional changes can be related to the increase of individual segments of DNA to a high number of copies, by incorporating or eliminating the inverted segments i.e. mobile DNA parts in code or regulatory gene flow, or inversion of DNA segments.

Mutations occur in the field in all living organisms spontaneously: both in those that reproduce only sexually or only asexually, and in those that reproduce both sexually and asexually. Mutations in fungi with a haploid mycelium can become apparent immediately after they occur. Most mutations are usually recessive. Therefore, in diploid or dicaryotic organisms, mutations may not become apparent while they are together in a diploid sporophyte.

As pathogens contain the genetic material (DNA) outside cell nuclei in the form of organelles or plasmids of DNA, or even as double-stranded RNA, mutations in extranuclear DNA are the same as in DNA in the nucleus and affect all characteristics controlled by extranuclear DNA. Through mutations in extrachromosomal DNA many pathogens gain (or lose) the ability to perform physiological processes which they could (or could not) perform previously. Cytoplasmic inheritance probably occurs in all organisms.

However, there are also some specific mechanisms of variability in pathogens, such as: heteroplody, heterokaryosis, euploidy and parasexualism (AGRIOS, 1997).

When individuals are formed asexually, the frequency and the degree of variability between the progeny is considerably lower, but even then some offspring individuals express different characteristics. As in micro-organisms a huge number of individuals are formed asexually, the total state of differences expressed by some fungi is great, perhaps even greater than the total differences in micro-organisms which reproduce sexually. This occurs in the asexual reproduction of fungi by conidia, zoospores, uredospores, then in bacteria and viruses (AGRIOS, 1997).

The results of this study can be compared to the results by other researchers who described similar phenomena in a great number of lignicolous fungi from the suborder *Basidiomycotina.* Based on the results, they were termed as incompatible physiological isolates for each fungus species. However, an adequate term has not yet been determined for this physiologically different isolates and zones of interaction. CHILDES (1937), who was the first to explain this phenomenon, used the term "demarcation line", and different races mycelia were termed "clones". ADAMS and ROTH (1967) report that many researchers describing the interaction between isolates used the terms "mutation aversion", "line of aversion" and "antagonism". BURNETT (1968) used the term "sterility barrier" and he classifies the species into inter-sterile groups in which the fusion of the members of different groups is prevented or reduced. According to the results reported by SCHMITZ (1925) and MOUNC (1929) for *Fomes (=Fomitopsis) pinicola*, VERRALL (1937) for *Fomes (=Phellinus) igniarius* and ADAMS and ROTH (1967) for *Fomes cajanderi*, it seems that in these species, but also in other species, there is a so-called "isolating mechanism" (BURNETT, 1968) which recognises the genetically distinctive mycelia.

In our study, the morphological differences between individual isolates are macroscopically distinctive. They are apparent by density, vigour and nuance of the mycelia. The mycelia, which can be distinguished based on the form of incompatibility, can be designated as "variety" genotypes - lines.

In the pairing of hyphae it is possible that two genetically conditioned phenomena act independently. The first one is active before fusion and it limits the pairing of heterogeneous hyphae. The second one is formed after fusion and results in the disorganisation of individual hyphae. Partial interactions between paired colonies indicate that genetic factors in some cells of the two confronted colonies are compatible and allow the fusion of hyphae, and in others they are not compatible and do not allow the fusion. So, the ratio of compatible and incompatible cells determines the density and width of the zone of interaction. The interaction behaviour of synthetic heterokaryons shows that they are formed by pairing of heterogeneous basidiospores and that they probably change the potential interaction of individual isolates.

CONCLUSION

Infection of felled beech log with basidiospores of the fungus *Fomitopsis pinicola* is massive, throughout the log length. This is followed by the development of conditionally termed, “variety” lines or “pathotypes” after the fusion of the mass of heterogeneous germinating basidiospores. The behaviour of recombined mycelia formed in this way is heterogeneous.

The study of interactions of the *F. pinicola* isolates formed by the fusion of different spores, shows the behaviour differences expressed in minimum three ways:

- fusion of isolates without interaction (5% mycelia pairs is classified in this category);
- initial fusion of isolates whose hyphae soon become dark in the entire zone of interaction (66% pairs is classified in this category);
- limited fusion of isolates whose hyphae form a narrow zone of interaction without discolouration (29% pairs is classified in this category);

Consequently, in 95% cases, the paired recombined isolates from different carpophores (22) and from the same carpophore (9) show some type of interaction. In 44% cases, the mycelia of paired isolates are morphologically different.

Based on the study results, it seems quite possible that the presence of permanent differences in the degree of interaction reflects the differences in the degree of compatibility between individual isolates. If this is that case, then it is possible that the zone of interaction is formed as the result of hyphae decomposition in the zone of initial fusion of mycelia. The mycelia which can be distinguished by the form of incompatibility can conditionally be termed “variety” lines, and their behaviour is very heterogeneous. These populations should also be distinguished by the genetic characteristics of the mycelia.

The zone of interaction or aversion is formed as the result of disorganisation of hyphae in contact zones of two incompatible colonies. In this study, the degree of aversion is observed through the density and width of interaction zone. The partial formation of interaction zone (aversion), as well as its density and width, can be explained by the ratio of compatible and incompatible cells in the sown colonies. The isolates of mycelia from the zone of interaction are the new “variety” lines which differ morphologically from both parental cultures, and regarding the compatibility of mycelia in the zone of interaction, they are compatible only with one parental culture.

The infection of felled beech trees by basidiospores of the fungus *Fomitopsis pinicola* is a mass phenomenon indicated by a great number of formed carpophores. The development of colonies on the artificial medium results in the fusion of heterogeneous germinating basidiospores, and their behaviour can be heterogeneous. Based on the laboratory study results, it can be concluded that the interaction of different, conditionally termed “variety” populations and physiological “races” (pathotypes) also occurs in the wood.

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INTERAKCIJE REKOMBINOVANIH MICELIJA GLJIVE *FOMITOPSIS PINICOLA* (SOW. EX FR.)KARST. NA PDA PODLOZI

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I z v o d

Infekcije oborenog bukovog drveta bazidosporama gljive *Fomotopsis pinicola* (Sow. ex Fr.) Karst. vrše se masovno čitavom dužinom trupca. Zatim započinje razvoj izdvojenih uslovno zvanih "varijetas" linija odnosno "patotipova" fuzijom mase raznorodnih klijajućih bazidiospora. Ponašanje raznih rekombinovanih micelija uzgajanih na veštačkoj hranljivoj podlozi (PDA) je heterogeno. Od 465 interakcijskih odnosa uporedno zasejanih rekombinovanih micelija *F. pinicola* iz različitih karpofora, pa i iste karpofore, utvrđene su razlike u njihovom ponašanju koje se ispoljavaju na najmanje tri načina:

-mešanje izolata bez interakcije (5 % parova micelije);

-početno mešanje izolata čije hife u čitavoj zoni interakcije brzo postaju tamne (66 % parova micelije);

-ograničano mešanje izolata čije hife obrazuju usku zonu interakcije koja se ne obojava (29 % parova micelije).

Dakle, u 95 % slučajeva spareni rekombinovani izolati iz različitih karpofora (22) i jedne karpofore (9) pokazuju neki tip interakcije. Na osnovu rezultata istraživanja u laboratoriji proizilazi da se interakcije različitih, uslovno nazvanih, ¨varijetas¨ linija i ¨fizioloških rasa¨ (patotipova) odvija i u drvetu.

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