

## **THE ABILITY TO GROW THE RHIZOMORPHS BY CHOSEN *ARMILLARIA OSTOYAE* (ROMAGN.) ISOLATES COMING FROM THE ZIELONKA FOREST DISTRICT AREA**

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**Abstract.** *Armillaria* root rot is one of the most dangerous infesting diseases of tree roots. *Armillaria* spp. spread and infest through rhizomorphs. Four samples were taken out and diploid mycelium was isolated. 28 isolates were chosen in order to examine rhizomorphs. The result of the experiment indicates the division of isolates from particular areas as well as within the areas.

**Key words:** *Armillaria ostoyae*, rhizomorphs

### **INTRODUCTION**

*Armillaria* root rot is one of the most dangerous infesting diseases of the roots, particularly in Scots pine plantations up to 20. It often occurs epidemic causing dying of the trees and creating gaps which are difficult to be productive again. Moreover the cultivation reaches density with difficulty and its transition into the young tree of 10-20 years' growth is passed. It has a negative impact on the formation of the stand and the structure of trees. *Armillaria* spp. spread and infect through rhizomorphs. That is the reason why the diagnosis of the ability to create rhizomorphs by various isolates coming from the stand being in danger of infection may indicate their real risk and direct the culture endeavors so that the losses which result in infecting the trees are reduced.

In the Zielonka Forest District permanent danger of *Armillaria* root rot occurs especially in young Scots pine plantations. During the last two decades the increase of the disease was observed [Mańka and Szewczyk 2000] what undoubtedly influences the growth of tree stand in the area. The aim of the work was to examine the ability to produce rhizomorphs by chosen *Armillaria ostoyae* isolates and to demonstrate whether the differences in the production of rhizomorphs among isolates from areas exist.

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## MATERIAL AND METHODS

5 isolates from division 47k, 7 isolates from division 85c, 6 isolates from division 64c and 10 isolates from division 43f were investigated in the experiment. All of the isolates of the diploid mycelium were obtained from rhizomorph coming from dead Scots pines on the investigated surfaces (Table 1).

Table 1. Characteristics and location of investigation plots  
Tabela 1. Charakterystyka i lokalizacja powierzchni badawczych

Division Wdzielenie	Forest Range Leśnictwo	Forest site type Typ siedliskowy lasu	Number of observation plots Liczba drzew na pow. obserwacyjnych	Pine age in 2002 Wiek sosny w 2002 roku	Number of dead trees in 1998-2002 Liczba zamarłych drzew latach 1998-2002
47k	Huta Pusta	BMśw	4×50	9	15
43f	Kamińsko	Bśw	4×50	12	21
64c	Kamińsko	LMśw	4×50	10	40
85c	Potasze	BMśw	4×50	9	29

In order to determine the ability of creating rhizomorphs by particular isolates of *A. ostoyae*, they were incubated in the temperature of 22°C for 28 days. In so obtained mycelium some disks cut out from oak sprouts (after earlier sterilization in the autoclave are hour for 2 days) were put. The disks were 2 cm in diameter – sterilized in the same way as the disks [Łakomy 1998]. The following stage was the insertion of so prepared inoculum in the jars with not sterile moist river sand. The jars were closed with aluminum foil and incubated for 14 weeks [Rishbeth 1982, 1984]. After this time the sprouts were taken out from the jars and placed in foil bags with the mixture of forest soil, litter and sand taken earlier from young Scots pine situated in the very proximity of the examined surfaces. The ingredients were mixed 1:1:1. After 10 weeks of storing in thermostat in the temperature of 23°C the sprouts were gently taken out so as not to damage the rhizomorph. Next they were measured in their length ( $d_{max}$ ), weight (m), the rhizomorph and their active endings were counted [Łakomy 1998].

The analysis for variations of all measurements specified above were conducted. In order to present the danger of infestation the formulas proposed by Rykowski [1985] were used.

A degree of rhizomorph's ramification (R) was counted:

$$R = \frac{n_s}{n_i}$$

where:

$n_i$  – number of rhizomorph produced,

$n_s$  – number of rhizomorph growing tops.

The values  $n_s$ ,  $d_{max}$ ,  $n_i$  and m obtained during the investigation were accepted as the base to show differences in rhizomorphs production in dependence on excepted variable.

However these data have to be related to the space in which the presence of rhizomorph carries the risk of infestation. Neither the same number of rhizomorphs' growing tops nor their weight determine comparable value when the volume of soil is varied. Rykowski [1985] introduced the following comparative criteria: an index of density of infestation (Y) is expressed with a number of growing rhizomorphs' tops (infesting units) in the unit of soil volume (1 cm<sup>3</sup>) penetrated by rhizomorphs. The index was counted with the following formula:

$$Y = k \frac{n_s}{l}$$

where:

- k – the ratio of proportionality (0.2387 = constans),
- $n_s$  – number of growing rhizomorph's tops,
- l – distance from the nutrient base (theoretically equal to the length of the longest rhizomorph).

The index of infesting potential (P) increase with the growth of infestation density (Y) and the infesting potential (P), in this connection:

$$Z = Y \times P \times 1000$$

## RESULTS

The average number of rhizomorphs amounted from 0 (isolate 017323r, 017324 – div. 64c, isolate 017334r – div. 85c isolate 017355r – div. 43f in which even though it was mycelium in shoot, the rhizomorphs did not occur) to 61 rhizomorph (isolate 017308 – div. 47k). A part from isolates, which did not produce rhizomorphs, the number of growing tops amounted from 5 (isolate 017342r – div. 43f) to 63 (isolate 017308r – div. 47k). In the case of isolate 017342r the number of rhizomorphs produced over lapses with the number of growing tops. The average mass amounted from 8 mg (isolates 017310r and 017344r) to 265 mg (isolates 017319r). Isolate nr 017319r characterized with the highest degree of rhizomorph branches, whereas the isolate 017330r characterized the highest index of infesting density therefore with the biggest degree of stuffing the soil with rhizomorphs, isolate 017330r (div. 64c). Isolate 017314r coming from the division 85c characterized the highest index of infesting potential and it amounted 9.8. Isolate 017330r, division 64c, characterized with the highest degree of infesting danger. Table 6 introduces the index of potential infesting danger caused by particular isolates *A. ostoyae*. The average number of produced rhizomorphs ( $n_i$ ), the average number of growing rhizomorph's tops ( $n_s$ ), the average rhizomorphs' mass (m), the average length of the longest rhizomorph ( $d_{max}$ ) the average length of shortest rhizomorphs' ( $d_{min}$ ), the average length of rhizomorphs ( $d_{average}$ ) produced by individual isolates *A. ostoyae* from individual divisions of the Zielonka Forest District is introduced in Table 1. By means of one-way analysis of variation, it was investigated whether the number of produced rhizomorphs is the same for particular isolates. The investigation was conducted for every surface separately and next for all surfaces together (Table 3).

Table 2. Average parameters of rhizomorph from individual division  
 Tabela 2. Średnie parametry ryzomorf z poszczególnych wydzielen

Division Wydzielenie	Isolate No Kod izolatu	$n_i$	$n_s$	m, mg	$d_{max}$ , cm	$d_{min}$ , cm	$d_{sr}$ , cm
47k	017305r	12	29	86	15.3	0.2	45.7
	017306r	13	30	108	14.8	0.4	21.9
	017308r	61	63	16	5.3	0.1	16.1
	017310r	7	11	8	2.2	0.9	9.7
	017311r	13	19	10	3.2	0.3	13.6
85c	017314r	8	20	196	35.8	19.5	66.9
	017315r	22	26	24	4.0	0.6	31.7
	017316r	10	19	42	1.8	0.2	22.4
	017319r	7	55	256	17.4	0.9	30.5
64c	017322r	16	17	20	4.0	1.2	28.8
	017323r	0	0	0	0.0	0.0	0.0
	017324r	0	0	0	0.0	0.0	0.0
	017328r	7	13	18	3.2	2.0	14.6
	017329r	14	16	30	3.8	0.3	9.9
	017330r	12	54	128	2.6	0.6	14.8
85c	017332r	5	14	58	3.1	0.9	10.1
	017333r	7	25	38	5.1	0.3	15.4
	017334r	0	0	0	0.0	0.0	0.0
43f	017340r	5	31	162	7.1	1.0	14.9
	017341r	4	17	94	6.4	0.8	13.8
	017342r	5	5	10	12.0	3.0	4.1
	017344r	3	4	8	1.3	0.4	2.9
	017346r	11	14	18	2.0	5.3	22.8
	017347r	39	55	80	11.4	0.8	94.7
	017349r	30	45	96	7.6	0.3	24.7
	017350r	11	18	44	2.4	0.2	8.9
	017351r	7	10	28	4.0	0.5	11.8
	017355r	0	0	0	0.0	0.0	0.0

$n_i$  – average number of rhizomorphs,  $n_s$  – average number of growing rhizomorph tips, m – average rhizomorph weight,  $d_{max}$  – average length of longest rhizomorph,  $d_{min}$  – average length of shortest rhizomorph,  $d_{sr}$  – average length of all rhizomorphs.

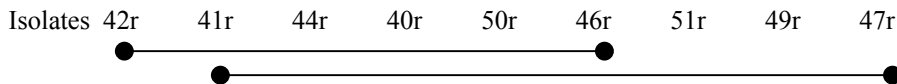
$n_i$  – średnia liczba wytworzonych ryzomorf,  $n_s$  – średnia liczba rosnących szczytów ryzomorf, m – średnia masa ryzomorf,  $d_{max}$  – średnia długość najdłuższych ryzomorf,  $d_{min}$  – średnia długość najkrótszych ryzomorf,  $d_{sr}$  – średnia długość ryzomorf.

Table 3. One-way ANOVA for influence isolate on the number of rhizomorphs  
 Tabela 3. Jednoczynnikowa analiza wariancji wpływu izolatu na liczbę ryzomorf

Source of variation Źródło wariancji	Sums of squares Sumy kwadratów	Degrees of freedom Stopnie swobody	Mean squares Średnie kwadraty	F calculated Empiryczna wartość statystyki F	p – value Wartość – p	Critical value the F-test ( $\alpha = 0.005$ ) Wartość krytyczna testu F ( $\alpha = 0,05$ )
Division 47k – Wydzielenie 47k						
Isolates Izolaty	0.2492	4	0.0623	1.1446	0.3644	2.8661
Error Błąd	1.0886	20	0.0544			
Total Ogółem	1.3377	24				
Division 85c – Wydzielenie 85c						
Isolates Izolaty	0.8509	5	0.1702	1.1792	0.3484	2.6207
Error Błąd	3.4635	24	0.1443			
Total Ogółem	4.3143	29				
Division 64c – Wydzielenie 64c						
Isolates Izolaty	1.3316	3	0.4439	1.4274	0.2716	3.2389
Error Błąd	4.9753	16	0.3110			
Total Ogółem	6.3069	19				
Division 43f – Wydzielenie 43f						
Isolates Izolaty	3.6523	8	0.4565	2.6608*	0.0210	2.2085
Error Błąd	6.1768	36	0.1716			
Total Ogółem	9.8291	44				
All divisions – Wszystkie wydzielenia						
Isolates Izolaty	9.4616	23	0.4114	2.5148**	0.0009	1.6423
Error Błąd	15.7040	96	0.1636			
Total Ogółem	25.1656	119				

In divisions 47k, 85c and 64c statistically important influence of isolate on the number of rhizomorphs does not exist. On the other hand in division 43f statistically important variation produced by rhizomorph exists.

Tukey's test:



Isolate 017342r produced intrinsically smaller number of rhizomorphs than isolates 017349r and 017347r. Having analyzed all the surfaces together one can come to the conclusion that highly intrinsic influence of isolate on the number of rhizomorphs exists.

Tukey's test:

Isolate	Average number of rhizomorph
017342r	1.0
017341r	2.6
017329r	2.8
017344r	3.0
017328r	4.2
017340r	4.2
017330r	4.6
017332r	5.4
017319r	5.4
017351r	6.2
017310r	7.2
017333r	7.4
017314r	7.8
017316r	9.8
017350r	10.6
017346r	10.8
017305r	12.4
017306r	12.6
017322r	16.2
017311r	19.4
017315r	21.6
017349r	29.8
017347r	39.0
017308r	60.6

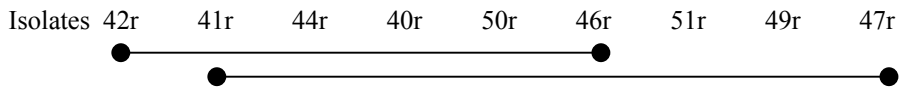
The number of rhizomorphs in isolates 017315r, 017347r, 017308r is statistically higher than in isolates 017342r and 017341r. By means of variation analysis it was investigated whether the number of growing rhizomorphs' tips for individual isolates is the same. The investigation was conducted for every individual investigative surfaces separately and then for all the surfaces together (Table 4).

Table 4. One-way ANOVA for influence of the isolate on the number of growing rhizomorph tips  
Tabela 4. Jednoczynnikowa analiza wariancji wpływu izolatu na liczbę rosnących szczytów ryzomorf

Source of variation Źródło wariancji	Sums of squares Sumy kwadratów	Degrees of freedom Stopnie swobody	Mean squares Średnie kwadraty	F calculated Empiryczna wartość statystyki F	p – value Wartość – p	Critical value the F-test ( $\alpha = 0.005$ ) Wartość krytyczna testu F ( $\alpha = 0,05$ )
Division 47k – Wydzielenie 47k						
Isolates Izolaty	0.2939	4	0.0735	1.3428	0.2888	2.8661
Error Błąd	1.0943	20	0.0547			
Total Ogółem	1.3881	24				
Division 85c – Wydzielenie 85c						
Isolates Izolaty	1.0331	5	0.2066	1.3041	0.2954	2.6207
Error Błąd	3.8028	24	0.1585			
Total Ogółem	4.8359	29				
Division 64c – Wydzielenie 64c						
Isolates Izolaty	1.3543	3	0.4514	1.3805	0.2847	3.2389
Error Błąd	5.2321	16	0.3270			
Total Ogółem	6.5863	19				
Division 43f – Wydzielenie 43f						
Isolates Izolaty	3.7776	8	0.4722	2.6067*	0.0233	2.2085
Error Błąd	6.5212	36	0.1811			
Total Ogółem	10.2987	44				
All divisions – Wszystkie wydzielenia						
Isolates Izolaty	10.1544	23	0.4415	2.5455**	0.0008	1.6423
Error Błąd	16.6503	96	0.1734			
Total Ogółem	26.8047	119				

In division 47k, 85c and 64c statistically important influence of the isolate on the number of growing rhizomorphs' tips produced by isolate exist.

Tukey's test for division 43f:



Isolate 017342r produced intrinsically smaller number of growing rhizomorph tips than isolates 017351r, 017349r and 017347r. Having analyzed all division together one can affirm essentially important influence of the isolate on the growing rhizomorph tips.

Tukey's test:

Isolate	Average number of growing rhizomorph tips
017342r	1.0
017329r	3.2
017344r	3.4
017328r	7.6
017351r	9.8
017341r	10.0
017310r	11.4
017346r	14.0
017332r	14.4
017322r	17.4
017350r	17.8
017316r	19.2
017311r	19.4
017330r	21.6
017333r	24.6
017340r	24.8
017315r	26.4
017305r	29.2
017306r	29.6
017319r	44.2
017349r	45.8
017314r	50.8
017347r	55.0
017308r	63.2

The number of growing rhizomorph tips in isolates 017314r, 017347r and 017308r is essentially higher than in isolates 017342 and 017329r. By means of variation analysis it was also investigated whether the mass of rhizomorphs is the same. The investigation was conducted for every division separately and then for all divisions together (Table 5).

There is no statistically essential influence of the isolates on the rhizomorph mass either for every investigative surface being analyzed separately and for all the surfaces together.



Table 5. One-way ANOVA for influence isolate for weight rhizomorphs  
 Tabela 5. Jednoczynnikowa analiza wariancji wpływu izolatu na masę ryzomorf

Source of variation Źródło wariancji	Sums of squares Sumy kwadratów	Degrees of freedom Stopnie swobody	Mean squares Średnie kwadraty	F calculated Empiryczna wartość statystyki F	p – value Wartość – p	Critical value the F-test ( $\alpha = 0.005$ ) Wartość krytyczna testu F ( $\alpha = 0,05$ )
Division 47k – Wydzielenie 47k						
Isolates Izolaty	45073.20	4	11268.3000	2.0890	0.1203	2.8661
Error Błąd	107884.80	20	5394.2400			
Total Ogółem	152958.00	24				
Division 85c – Wydzielenie 85c						
Isolates Izolaty	172882.67	5	34576.5333	1.5429	0.2142	2.6207
Error Błąd	537835.20	24	22409.8000			
Total Ogółem	710717.87	29				
Division 64c – Wydzielenie 64c						
Isolates Izolaty	10426.95	3	3475.6500	0.5575	0.6506	3.2389
Error Błąd	99748.00	16	6234.2500			
Total Ogółem	110174.95	19				
Division 43f – Wydzielenie 43f						
Isolates Izolaty	74378.44	8	9297.3056	0.8290	0.5830	2.2085
Error Błąd	403752.00	36	11215.3333			
Total Ogółem	478130.44	44				
All divisions – Wszystkie wydzielenia						
Isolates Izolaty	361942.3667	23	15736.6246	1.3146	0.1791	1.6423
Error Błąd	1149220.0000	96	11971.0417			
Total Ogółem	1511162.3667	119				

Table 6. Ratio of potential infection risk due to infection by *Armillaria ostoyae* isolates from division Zielonka DistrictTabela 6. Wskaźniki potencjalnego zagrożenia infekcyjnego przez poszczególne izolaty *Armillaria ostoyae* z badanych wydziałów Nadleśnictwa Zielonka

Division Wydziałenie	Isolate No Nr izolatu	R	Y	P	Z	a, cm
47k	017305r	2.42	0.4524	2.9655	1341.59	0.6781
	017306r	2.30	0.4838	3.6000	1741.68	0.6449
	017308r	1.05	2.8373	0.2539	720.39	0.1593
	017310r	1.57	1.1935	0.7272	867.91	0.1583
	017311r	1.46	1.4172	0.5263	745.87	0.1752
85c	017314r	2.50	0.1333	9.8000	1306.34	1.9108
	017315r	1.16	1.5515	0.9230	3433.46	0.1872
	017316r	1.90	2.5196	2.2105	5569.57	0.0985
	017319r	7.86	0.7545	4.6545	3511.82	0.5600
64c	017322r	1.06	1.0144	1.1764	1193.34	0.2315
	017323r	0	0	0	0	0
	017324r	0	0	0	0	0
	017328r	1.86	0.9697	1.3846	1342.64	0.2118
	017329r	1.14	1.0050	1.8750	1391.52	0.2267
	017330r	4.50	4.9576	2.3703	11750.99	0.0844
85c	017332r	2.80	1.0779	4.1428	4465.52	0.1977
	017333r	3.57	1.1700	1.5200	1778.40	0.2434
	017334r	0	0	0	0	0
43f	017340r	6.20	1.0422	5.2258	5340.77	0.3043
	017341r	4.25	0.6340	5.5294	35056.40	0.3705
	017342r	1.00	0.0994	2.0000	198.80	1.2809
	017344r	1.30	0.7344	2.0000	2937.60	0.1551
	017346r	1.27	1.1516	1.2857	1480.61	0.1275
	017347r	1.41	1.4133	1.4545	2055.64	0.3669
	017349r	1.50	1.4133	2.1333	3014.99	0.2704
	017350r	1.64	1.7902	2.4444	4379.64	0.1350
	017351r	1.43	1.4918	2.8000	4177.04	0.3019
	017355r	0	0	0	0	0

R – degree of rhizomorph branching, Y – the ratio of the infection density, P – the ratio of the infection potential, Z – degree of the infection risk, a – modulus of the infection density.

R – stopień rozgałęzienia ryzomorf, Y – wskaźnik gęstości infekcji, P – wskaźnik potencjału infekcyjnego, Z – stopień zagrożenia infekcyjnego, a – moduł gęstości infekcji.

## DISCUSSION AND CONCLUSIONS

The stumps obtained after removed stand are the nutrition base in phase of saprotrophic growth *A. ostoyae*, and the quantity and the quality of the base essentially influence the parasitic phase [Zycha 1970]. In the stump of big diameter it can exist and produce rhizomorphs from 30 to 70 years [Redfern and Filip 1991, Rishbeth 1978]. According to Pronos and Patton [1978] the largest number of rhizomorphs was produced after 10 years from elimination of the oak stand. It is possible that deciduous stumps wood consists of substances which stimulate forming of rhizomorph and coniferous wood inhibits this process [Termorshuizen 2000]. Conducted experiment indicates differentiation of isolates among divisions in terms of produced rhizomorphs and the number of active endings. If one considers the variation on the investigative surfaces, statistical variations occurred only on one division 43f. If one considers rhizomorphs mass, they did not vary statistically either on surfaces or among surfaces. Results obtained by Łakomy [1998], who analyzed *Armillaria ostoyae* isolates coming from one division as well as among divisions showed differentiation of all the parameters discussed. According to same research isolates of a given species *Armillaria ostoyae* may represent different genotypes.

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**ZDOLNOŚĆ WYTWARZANIA RYZOMORF PRZEZ WYBRANE IZOLATY  
*ARMILLARIA OSTOYAE* (ROMAGN.) POCHODZĄCE Z TERENU  
NADLEŚNICTWA ZIELONKA**

**Streszczenie.** Opieńkowa zgnilizna korzeni (*Armillaria* spp.) jest jedną z najgroźniejszych chorób infekcyjnych korzeni drzew. Grzyby rodzaju *Armillaria* rozprzestrzeniają się i dokonują infekcji za pomocą ryzomorf. Z terenu pięciu wydzieleń Nadleśnictwa Zielonka pobrano próby a następnie wyizolowano grzybnię diploidaną. Do badania zdolności wytwarzania ryzomorf wybrano 28 izolatów. Wynik doświadczenia wskazuje zarówno na zróżnicowanie izolatów z poszczególnych powierzchni, jak również między powierzchniami.

**Słowa kluczowe:** *Armillaria ostoyae*, opieńka, ryzomorfy, zagrożenie infekcyjne

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