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Studies of morphological structures of *Monilia coryli*

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A considerable morphological differentiation within the population of *Monilia coryli* was found. Both the strains forming macroconidia and strains forming microconidia on phialids were observed. Clusters of phialids and microconidia formed spherical aggregates. Strains cultivated on PDA and PDA with yeast extract after 21–28 days formed macroconidia, which made it possible to identify the species. Sometimes the strains formed also sclerotia, which did not produce apothecia but only sporodochia of *M. coryli* consisting of conidiophores and conidia.

Key words: *Monilia coryli*, culture, morphological structures.

INTRODUCTION

Recent studies on the healthiness of hazel plantations in the region of Lublin indicated frequent occurrence of a moniliosis caused by *Monilia coryli* (Machowicz-Stefaniak and Zalewska 2000). The disease can be readily recognized directly on the plant, on the basis of sporodochia formed on the mummified nuts. The absence of such symptoms on inflorescences and fruitlets as well as the morphological differentiation of the strains within the population of *M. coryli* makes it difficult to identify the pathogen (Machowicz-Stefaniak and Zalewska 2000).

The purpose of the present work was to study the morphological differentiation of strains of *M. coryli* obtained from different parts of reproductive organs of hazel and to learn the circumstances affecting the production of morphological structures of the fungus.

MATERIAL AND METHODS

The strains of *Monilia coryli* isolated in 1994–1997 from male and female inflorescences, fruitlets and unripe and ripe fruits of hazel were studied.

The material was isolated from artificial cultures on malt-agar medium after the method described in our preceding paper (Machowicz-Stefaniak and Zalewska 2000).

Non-sporulating strains of *M. coryli* L 1833, L 1838, L 1843, L 1845, L 1847, L 1850, L 1851 L 1853, L 1857, L 1867 were incubated on different media poor-agar, malt-agar, salt-agar, Czapek-Dox, PDA, PDA with yeast extract at 24°C for 48 days. Mycelial circlets (3 mm diameter) of *Monilia coryli* strains taken from 5-day-old colonies were placed in the centre of 90 mm Petri dishes. Four replications were used for each strain. Observations on the growth and morphological structures produced by the strains were made. In order to determine the influence of temperature on the production of macroconidia, the strains were cultivated on PDA medium at 2°C, 7°C, 14°C, 22°C, 27°C and 32°C in the way described above. The strains producing nothing but sclerotia and microconidia through several weeks of cultivation were exposed to a procedure after Lorenz and Eichhorn (1983) to obtain the perfect stage of the fungus. Thus, the strains were maintained without light first at 0°C for 4 weeks and next at 5°C for 2 weeks. Sclerotia obtained this way were placed in six sterilized Petri dishes half-filled with quartz sand (5 sclerotia per dish) and then treated with highly concentrated water-suspension of microconidia (0.01 ml of the suspension per sclerotium). Other six Petri dishes containing sclerotia treated with sterile distilled water were the control. Sclerotia slightly covered with sand and treated with sterile water were incubated without light at 15°C for 4 weeks. Afterwards, the sclerotia were exposed to day light to germinate at last.

RESULTS AND DISCUSSION

Within the population of *Monilia coryli* two groups of strains could be distinguished: these forming macroconidia immediately after isolation, and those forming nothing but microconidia (Table 1). The strains belonging to the first group were isolated from fruitlets and unripe nuts of hazel in rather warm and moderately humid seasons of 1994 and 1995 (Tables 1 and 2). The colonies of such strains on culture media were usually cream-white, fluffy and rich in sporodochia. The strains isolated from male and female inflorescence and the strains isolated from unripe fruits of hazel during exceptionally adverse weather conditions (cold July of 1996 and rainy July of 1997) formed not only macroconidia but also microconidia (Tables 1 and 2).

The colonies of the strains belonging to the second group (i.e. forming only microconidia immediately after isolation) were, unlike those forming macroconidia, flat, grey-white and almost black on their reverse sides whereas mycelial filaments formed a velvet mat. The microconidia were formed in the range of temperatures from 7°C to 32°C, yet at 7°C and 32°C they appeared at the earliest, i.e. after 10 days of cultivating. The microconidia

Table 1

The occurrence of *Monilia coryli* on the organs of hazel studied in 1994–1997

Species of fungi	Number (%) of isolates																			
	male inflorescences				female inflorescences				fruitlets				unripe fruit				ripe fruit			
	1994	1995	1996	1997	1994	1995	1996	1997	1994	1995	1996	1997	1994	1995	1996	1997	1994	1995	1996	1997
Monilia coryli (strains with macroconidia)	–	1 (0.2)	1 (1.2)	–	–	7 (2.5)	1 (2.4)	–	18 (6.5)	37 (11.6)	–	6 (3.4)	60 (26.4)	97 (20.1)	35 (9.6)	12 (2.9)	–	–	–	10 (1.4)
Monilia coryli (strains with microconidia)	–	167 (39.6)	–	–	–	27 (9.6)	–	–		–	–	3 (1.7)	–	–	20 (5.5)	72 (17.6)	–	11 (0.9)	11 (1.4)	–
Other species	76	254	82	211	248	248	40	156	258	281	78	169	167	386	310	326	162	1176	764	689
Total	76 (100)	422	83	211	248	282	41	156	276	318	78	178	227	483	365	410	162	1187	775	699

Table 2

Air temperatures and rainfall in vegetation season in the years 1994–1997

Months	Means for the years 1951–1997		Difference of air temperatures in comparison with mean				Percentage of the rainfall norm			
	air temp. in C	rainfall in mm	1994	1995	1996	1997	1994	1995	1996	1997
January	−3.8	26.2	5.2	1.5	−3.1*	−2.3*	169.0	44.4	34.6	5.3
February	−2.9	27.3	0.0	5.2	−3.9*	3.1	73.0	11.0	89.6	41.6
March	1.1	27.1	0,2	1.2	−4.1*	0.7	214.0	172.0	84.8	55.8
April	7.4	40.4	1.5	0.0	−0.1*	−3.5*	198.0	102.3	36.6	97.1
May	13.3	54.4	−0.7*	−1.0*	2.3	0.7	97.0	57.3	226.4	162.9
June	16.5	68.9	0.7	0.6	0.0	−1.1*	19.0	106.7	39.4	50.9
July	18.0	78.3	2.9	1.8	−1.6*	1.8	22.0	34.6	99.0	224.1
August	17.1	73.7	0.9	2.1	0.6	1.1	129.0	87.3	120.4	45.0
September	12.9	47.3	2.5	0.0	−3.5*	0.4	122.0	220.2	185.1	105.3
October	7.8	41.0	−1.6*	1.8	0.6	−1.7*	199.0	27.0	135.7	263.8
November	2.5	40.9	0.3	−3.4*	3.0	−0.2*	66.0	58.5	155.2	87.0
December	−1.4	33.8	1.0	−4.0*	−4.4*	−0.4*	129.0	45.0	42.2	82.0

* – temperature lower than mean for the years

were spherical, colourless, 1.5–2.8 mm in diameter (Fig. 1), fixed on the top of phialids, which were 3.7–9.25 μm in length. The phialids were formed directly on the cells of mycelial filaments or on short, up to 9.25 μm in length basal cells shooting from the primary filament. At first they appeared one by one, in groups of two or three, and afterwards the phialids, present on the cultures, which were growing old, formed numerous bushy clusters made of more than ten phialids. The clusters of phialids and microconidia associated together formed spherical aggregates measuring from 37.1 μm to 63.0 μm (Fig. 1).

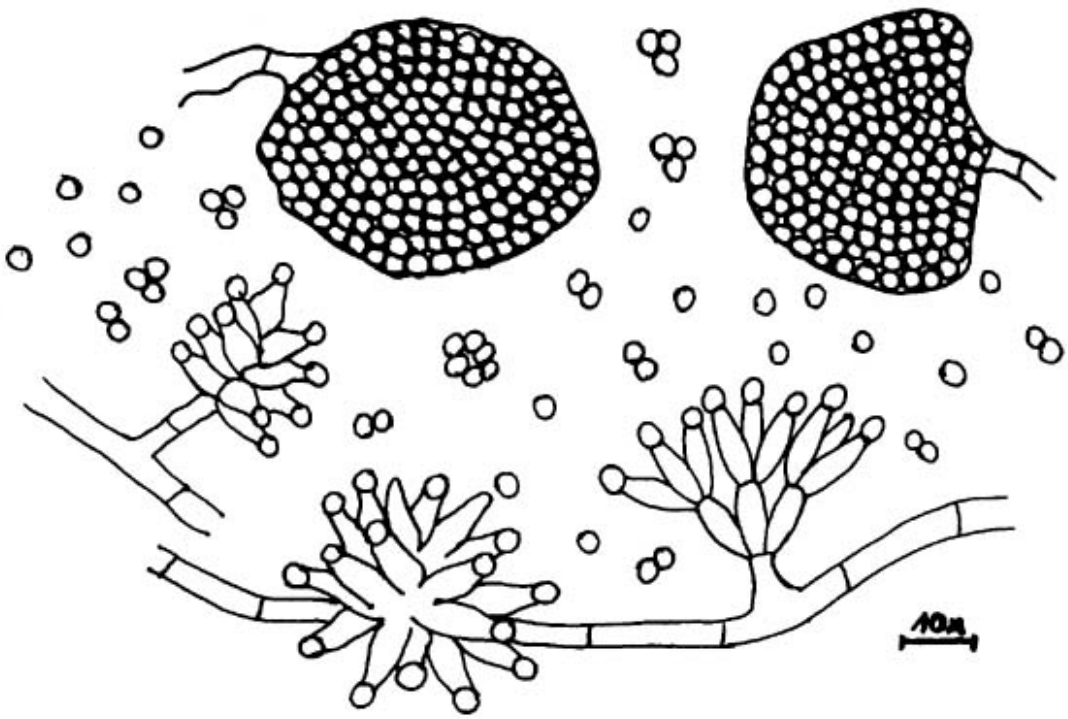
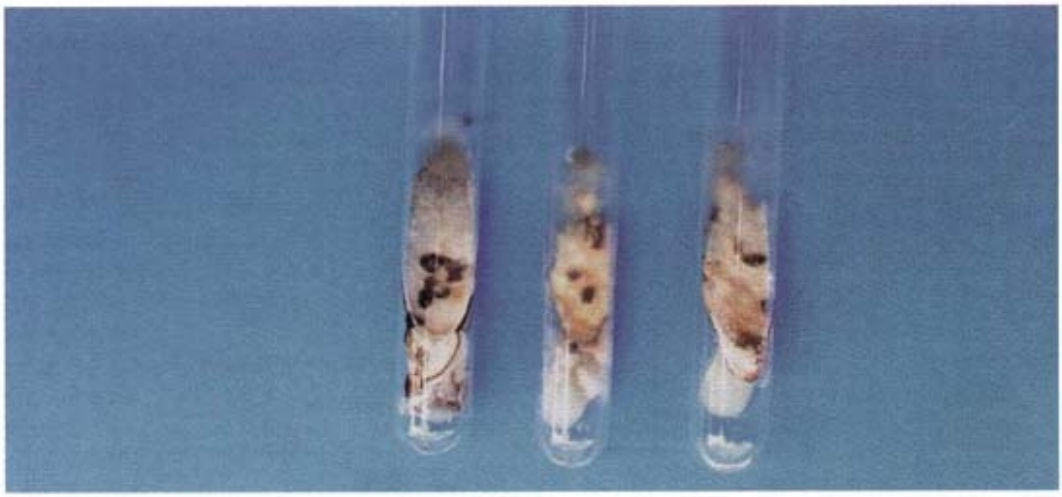


Fig. 1. Microconidia and aggregates of microconidia of *Monilia coryli*, strain L 659

The strains under discussion, although cultivated on poor-agar, Czapek-Dox medium and mineral medium at 24°C, did not form sporodochia with macroconidia by the 42nd day of cultivation (Table 3). The morphological structures described above appeared in the majority of such strains not before 24–28 days of growth on the media PDA and PDA with yeast, and after 30–40 days of growth on maltose medium (Table 3). The macroconidia present in the cultures made it possible to identify the species of the pathogen. Some strains cultivated on PDA and PDA with yeasts for 18–42 days yielded sclerotia, which rarely appear in *Monilia* spp. and which proved similar to those of *Botryotinia fuckeliana* (Byrde and Willets 1977; Jarvis 1977; Machowicz-Stefaniak 1998).



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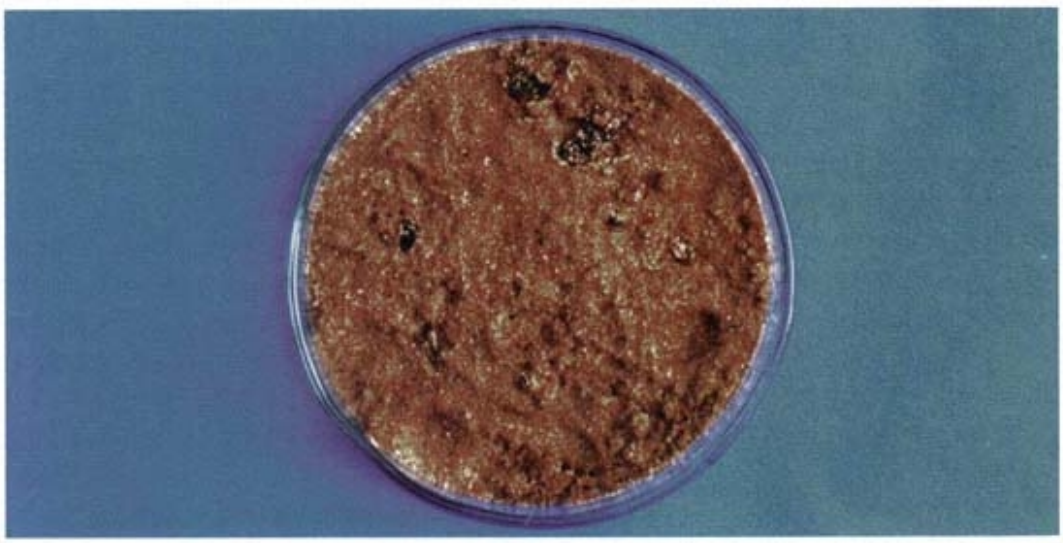

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Figs 2–4. Fig. 2. Sclerotia of *M. coryli* on PDA medium. Fig. 3. Sclerotia of *M. coryli* on sterile quartz sand. Fig. 4. Sporodochia of *M. coryli* formed on germinating sclerotia

Table 3
The effect of medium on the production of morphological structures of *Monilia coryli* (data for 10 strains producing microconidia at 24°C)

Morphological structures	Medium/day of appearrance of morphological structures					
	PDA	PDA with yeast extract	malt-agar	poor-agar	Czapek--Dox	salt-agar
Microconidia	14th day	14th day	14th day	14th day	28th day	21st day
Aggregates of microconidia	14th day	14th day	14th day	14th day	absent	absent
Sclerotia	18th–42nd day	18th–42nd day	absent	absent	absent	absent
Sporodochia	28th day	21st day	30th–35th day	absent	absent	absent
Macroconidia	28th day	21st day	35th–40th day	absent	absent	absent

Sclerotia originating from the cultures of *Monilia coryli* (Fig. 2) treated with highly concentrated water-suspension of microconidia began to germinate 6 months after being kept in sterile quartz sand, i.e. in the beginning of March 1997 (Figs 3 and 4). On their surface sporodochia were formed one by one or in groups, 2–4 mm in height and of beige colour (Fig. 4), containing conidial stalks and macroconidia in shape and dimensions characteristic of *Monilia coryli*. No apothecia were formed on the sclerotia examined by the 18th month of observations. The perfect stage of *Monilinia* spp. is known to occur occasionally (Byrde and Willets 1977; Batra 1983; Batra and Harada 1986) and therefore this stage, on the contrary to the conidial stage, is of no importance to the taxonomy of *Monilia coryli*.

Morphological differentiation of the populations of *Monilia coryli*, in particular of those obtained from generative organs of hazel in early spring, resulted in two groups of strains, sporulating intensively and non-sporulating. This might be the reason why the appearance of this species was overlooked as just macroconidia are needed to distinguish the species (Byrde and Willets 1977). A possibility of producing microconidia by *Monilia* spp. and other representatives of *Sclerotiniaceae* was indicated by Byrde and Willets (1977), Jarvis (1977), and Urbasch (1984). Frequent isolations of the strains forming microconidia in early spring as well as in the cold and rainy summer of the 1996 and 1997 suggest that microconidia are formed under the conditions regarded as unfavourable to the development of *M. coryli*. This was confirmed by the fact that microconidia were formed faster by the strains cultivated on artificial media at the temperatures of 7°C, 14°C and 32°C than at those of 22°C and 27°C. It seems that the formation of microconidia, aggregates of microconidia, and sclerotia may be associated also with ageing of the colonies of the fungus as well as with a deficiency of nutrients and with the abundance of toxic products of metabolism of the fungus present in the medium. These substances may cause the autolysis of cells

and darkening of the mycelium, which is accompanied by the formation of microconidia (W i l l e t s 1969, according to quoted literature). The occurrence of the so-called aggregates of microconidia most probably extends the vitality of the fungus as was experimentally shown in *Botrytis cinerea* by U r b a s c h (1984).

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Badania struktur morfologicznych *Monilia coryli* Schellenb.

S t r e s z c z e n i e

W pracy badano zróżnicowanie morfologiczne szczepów *Monilia coryli* oraz warunki tworzenia się struktur morfologicznych grzyba. W analizowanej populacji *M. coryli* wyróżniono dwie grupy szczepów: wytwarzające bezpośrednio po izolacji makrokonidia oraz szczepy wytwarzające tylko mikrokonidia. Kolonie szczepów z makrokonidiami były na podłożach hodowlanych biało kremowe, puszyste z licznymi sporodochiami i kremowym rewersem. Kolonie tworzące tylko mikrokonidia były płaskie, zwarte, szaro-białe z prawie czarnym rewersem. Mikrokonidia tworzyły się w zakresie temperatury od 7°C do 32°C, przy czym w temperaturze 7°C i 32°C pojawiały się najwcześniej. Kuliste, bezbarwne mikrokonidia powstawały pojedynczo na szczycie fialid. Skupienia fialid wraz z tworzącymi się na nich mikrokonidiami przybierały kształt kulistych agregatów. Takie szczepy po 21–28 dniach w temperaturze 24°C na PDA i PDA z dodatkiem drożdży wytworzyły makrokonidia, dające podstawę do identyfikacji gatunku, a niekiedy sklerocja rzadko notowane u *Monilia* spp. Na badanych sklerocjach nie uzyskano apotecjów *Monilinia coryli*, a jedynie sporodochia złożone z trzonków konidialnych i konidiów *Monilia coryli*. Sporadyczne tworzenie stadium doskonałego przez *Monilinia* spp. wskazuje, że nie ma ono w przeciwieństwie do stadium konidialnego, znaczenia przy taksonomii omawianego gatunku.