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Fungal colonization of tobacco waste

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Tobacco dust colonization by soil fungi involves the succession of physiologically differentiated groups. They are characterized by poorly diversified species composition and are dominated by potentially phytopathogenic forms.

Key words: fungi, tobacco dust, colonization, soil, horticultural substrates.

INTRODUCTION

Tobacco dust is one of the most attractive means of fertilization due to is high level of nitrogen. Up to now, agricultural utilization of that waste has been primitive. It is based on a direct introduction into the soil. However, such introduction of non-processed organic matter may have a negative effect on the soil environment (I i m e n e z and G a r c i a 1989; M y ś k ó w 1989). Changes leading to naturally shaped microbiocenotic balance and accumulation of phytopathogenic forms of microorganisms are particularly unfavourable (M y ś k ó w 1989). Earlier studies (K o r n i ł ł o w i c z-K o w a l s k a, S z w e d and G o s t k o w s k a 1999) showed that the introduction of crude tobacco dust into the soil changed the relation between populations of potential antagonists and phytopathogens with preference for *Fusarium* individuals.

In the present paper, the species composition of fungi colonizing tobacco dust in the soil with special attention being paid to the above mentioned populations of *micromycetes* was investigated.

MATERIAL AND METHODS

Tobacco dust obtained from the Tobacco Plant in Lublin was investigated. Characteristics of the waste are given in an earlier work (K o r n i ł ł o w i c z - K o w a l s k a et al. 1999). Sandy soil taken from filed and horticultural substrates (universal soil) purchased from a store were used in the experiments. Such a choice of the soil material was made due to the differentiation of their chemical properties (Table 1).

T a b l e 1
Some chemical properties of the studied soil and horticultural substrates

Object	C org.	N total	pH_{KCl}
Sandy podzolic soil developed from loamy sands	0.302	0.039	3.97
Horticultural substrates	9.97	1.36	6.94

Soil samples were taken as described previously (K o r n i ł ł o w i c z - - K o w a l s k a et al. 1999). After averaging and screening trough the sieve with 2-mm diameter mesh, samples were enriched with 2% tobacco dust addition (recalculated into organic matter). After mixing, 10 kg samples were incubated for a month at the room temperature and mixed every week. Subsequently, the samples were placed into the 1000 cm^3 sterile vessels to get a soil layer of ca 25 cm. After the humidity had been adjusted up to 50–60 t.w.c., two caprone bags filled with tobacco dust (10 g each) were introduced. Six samples were made for each combination. The soil and horticultural medium without tobacco dust addition were the control. The incubation of samples was carried out at 20°C±2°C. Periodically (after 2, 5 and 10 weeks) the bags were removed and subjected to mycological analysis. The fungi were isolated on Martin's and Sabouraud's media with actidione as well as on Winogradzki's medium with cellulose as the only carbon and energy source. All the media were prepared on tobacco extracts. Streptomycin and chloromycetin in the same amount as for Martin's medium were applied to each sample to stop the growth of bacteria.

The isolation of fungi colonizing tobacco dust was carried out using the pellets layout technique: 20–25 for each repetition. Species composition in the controls was determined by means of the dilution plating method – all grown colonies were split off from two (out of five) dish repetitions.

Fungal isolates were identified on the base of macro- and micromorphological features observed on dishes and in microcultures the final classification was made according to: D o m s c h, G a m s and A n d e r s o n (1980), K w a ś n a, C h e ł k o w s k i and Z a j k o w s k i (1991), and N e l s o n, T o u s s o n and M a r a s a s (1983).

RESULTS AND DISCUSSION

The present study showed that fungal propagules were absent from crude tobacco dust. Among 300 investigated dust pellets, no fungi were found. The lack of fungi in the material studied is undoubtedly attributed to thermal preparation of tobacco raw material during technological processes as well as storage in a dried form. Before processing, the tobacco is inhabited by a community of different fungi (F l o r c z a k 1997).

The introduction of tobacco dust into the soil and horticultural medium resulted in its fast colonization by fungi indigenous to these environments (Table 2). The colonization rate was greater in the soil than in the horticultural substrates. This was due to the lower content of organic matter in the soil.

T a b l e 2

Fungal colonization of tobacco dust in arable soil (A) and horticultural substrates (B)

Medium	Time of cultivation (weeks)	Number of soil ground	Percentage of colonization		Number of:					
					strains		species		genera	
			A	B	A	B	A	B	A	B
Martin's	2	150	54	33	80	49	4	3	4	3
	5	120	100	100	183	155	10	7	4	4
	10	120	100	100	206	157	9	8	6	4
Sabouraud's	2	150	33	27	50	40	4	3	4	4
	5	120	100	100	160	224	6	5	4	4
	10	120	100	100	174	233	5	5	4	4
Winogradzki's with cellulose	2	150	9	12	14	18	2	2	2	2
	5	120	43	69	52	83	3	3	3	3
	10	120	98	100	117	124	3	3	3	2

The colonization of tobacco dust by fungi was of successional and nutritive character. Sugar fungi representing the genus *Mucor* appeared first (Figs 1 and 2). Significant content of sugars in a tobacco material (B e r b e ć et al. 1994) determined domination of these fungi by the 5 week after introduction into the soil and horticultural substrates. At that time an increased number of *Fusarium* propagules was observed. The high frequency of *Fusarium* species in the initial stage of dust colonization might have also been determined by the level of sugar fraction. Sugars are those organic compounds that *Fusarium* species (usually pathogenic forms) make use of under natural conditions (K w a ś n a et al. 1991). From the 5th week of study, *Fusarium* species were also isolated in a great number from the medium with cellulose (Figs 1 and 2). Therefore if may be assumed that after exhausting all easily available sugars, cellulose was one of the organic carbon sources stimulating fungal growth.

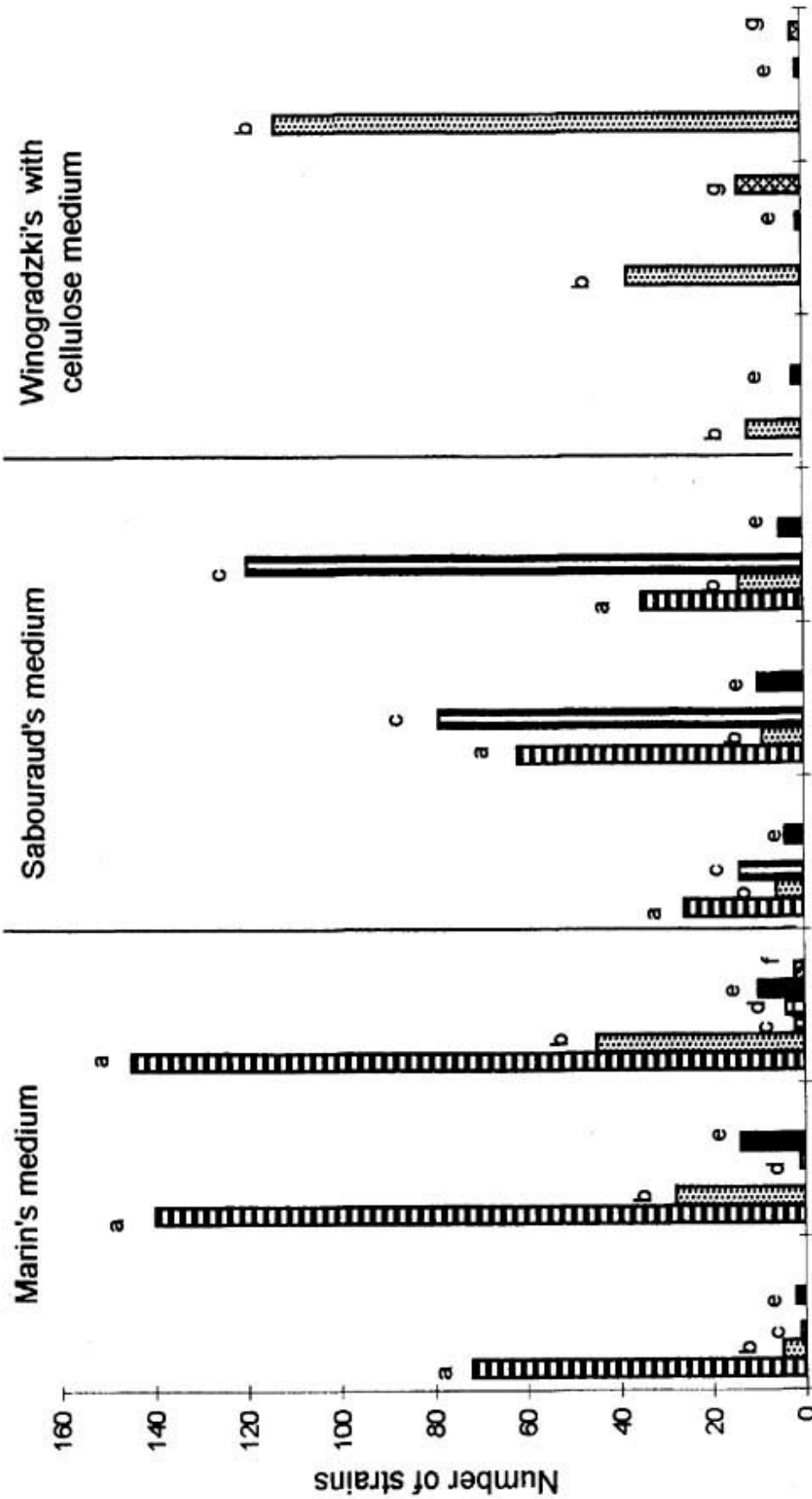

Fig. 1. The frequency of occurrence of some fungi genera that colonize tobacco dust in arable soil. a – *Mucor*, b – *Fusarium*, c – *Geotrichum*, d – *Gliocladium*, e – *Penicillium*, f – *Botrytis*, g – *Scopulariopsis*

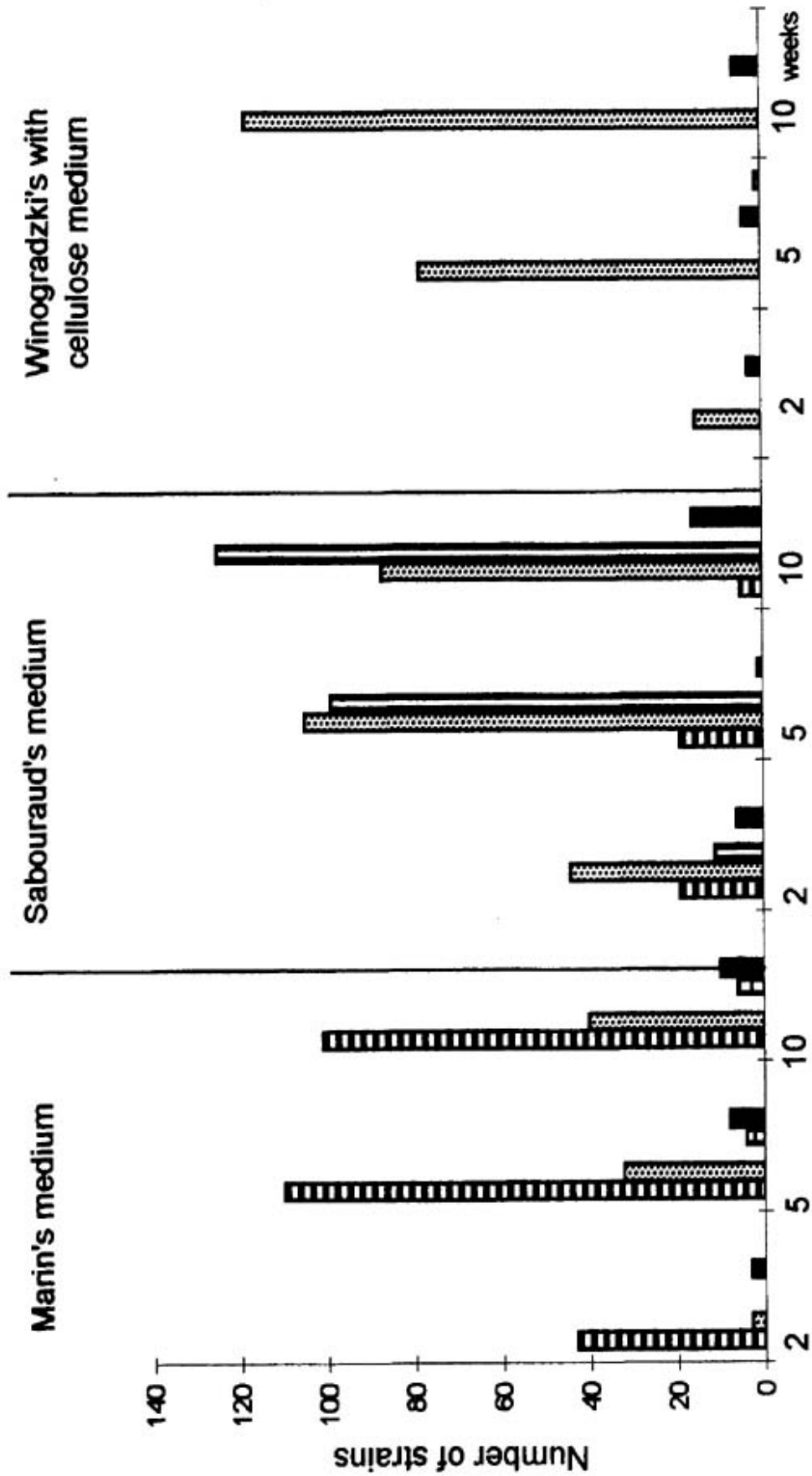

Fig. 2. The frequency of occurrence of some genera colonizing tobacco dust in horticultural substrates. Explanations see in Fig. 1.

Along with an increase in the density of celluloytic *Fusarium* species on tobacco dust, the number of non-cellulolityc species of *Geotrichum* also increased (D o m s c h et al. 1980) (Figs 1 and 2). The maximum growth of *Geotrichum* populations was recorded in the final stage of the study, i.e. after the 10 week from dust introduction into the soil and horticultural medium. *Geotrichum* was first of all isolated on Sabouard's medium with actidione. It is known that *Geotrichum* species can utilise various LMW phenols easily (D o m s c h et al. 1980). They are also active in decomposing the after-vanillin lignin and the so-called black liquor-lignin wastes arising from cellulose paper production (M a l a r c z y k, K o r n i ł ł o w i c z and L e o n o w i c z 1998). Therefore it may be assumed that *Geotrichum* population was involved in degradation of phenolic compounds of tobacco wastes. The content of polyphenols and lignocellulose in tobacco raw material ranges from 4 to 17 per cents and from 5 to 25 per cents, respectively (S k i e n d z i e l e w s k i and B i s k u p 1966; T r z c i ń s k i 1952). Moreover, it seems that monosaccharides released during the decomposition of cellulose and non-cellulose glucans present in tobacco dust were additional factors favouring the growth of *Geotrichum* species. M a l a r c z y k et al. (1998) found that some lignin wastes were decomposed by *Geotrichum* species only in the presence of glucose.

The effect of tobacco dust of *micromycetes* of both studied environments was strongly selective. In spite of great species diversity of fungi in the soil and horticultural substrates, tobacco dust was colonizing almost exclusively by *Mucor ramonissimus*, *Fusarium solani*, *F. oxysporum*, *F. redolens* and *Geotrichum candidum*. The above species (except for *M. ramonissimus*) were not common in the soil and horticultural substrates (Table 3). Apart from *M. ramonissimus*, recognized as a saprotroph, the three latter species represented potentially phytopathogenic forms. Their presence on tobacco dust is not unusual. Pathogenic strains of these species, especially of *F. solani* and *G. candidum* (B u t l e r 1960, K w a ś n a et al. 1991) often infect the plants from the *Solanaceae* family. Another factor favouring the growth of these fungi could be the lack of their natural antagonists, e.g. *Trichoderma viride*. This fungus efficiently reduces the colonization of organic matter in the soil by *F. solani* (K o r n i ł ł o w i c z-K o w a l s k a 1991/1992).

The accumulation of propagules of potentially pathogenic fungi on tobacco dust resulted in the formation of unfavourable biotic system, as the phytosanitary state is concerned (Table 4). The system was much more favourable in the soil and horticultural substrates, since it was dominated by potential antagonists (Table 4). The development of potentially phytopathogenic fungi on tobacco dust was undoubted the reason for changes in the relationships between the populations of phytopathogens and potential antagonists after soil enrichment with crude tobacco dust, which was observed in previous studies (K o r n i ł ł o w i c z-K o w a l s k a et al. 1999). Thus, it seems that supplying the soil with raw tobacco dust, resulting in the development of potentially pathogenic fungi, may have a negative effect on the growth and development of plants.

Table 3
Species composition of fungi colonizing tobacco dust in the soil and horticultural substrates

Species of fungi	Tobacco dust in:		Soil	Horticultural substrates
	soil	horticultural substrates		
Aspergillus niger van Tieghem	–	–	+(1)	–
Beauveria brongniartii Petch (Sacc.) Petch	–	–	–	+(2)
* *Botrytis cinerea* Pers: Fr.	+(1)***	–	+(2)	–
Chaetomium cochlioides Pall.	–	–	+(1)	–
Chaetomium sp.	–	–	–	+(4)
Chrysosporium pannorum Link (Hughes)	–	–	–	+(15)
Cladosporium cladosporioides (Fres.) de Vries	–	–	+(5)	–
* *Fusarium culmorum* (W.G.Sn.) Sacc.	–	–	+(4)	–
* *F. oxysporum* Schlecht.	+(27)	+(28)	+(1)	+(3)
* *F. solani* (Mart.) Sacc.	+(192)	+(24)	+(1)	+(2)
* *F. redolens* Wollenw.	+(52)	+(15)	–	+(1)
Geotrichum candidum Link ex. Leman	+(215)	+(225)	–	–
** *Gliocladium catenulatum* Gilm. et Abbott	–	–	+(1)	–
** *G. roseum* Bain.	+(5)	+(10)	+(1)	+(1)
** *G. virens* Miller, Giddens et Foster	–	–	+(1)	–
Gonatobotrys simplex Corda	–	–	–	+(1)
Humicola fuscoatra Traaen	–	–	–	+(1)
H. grisea Traaen	–	–	+(1)	+(1)
Metarrhizium anisopliae (Metschn.) Sorok.	–	–	–	+(1)
Mortiella alpina Peyronel	–	–	+(2)	–
M. vinacea Dixon-Steward	–	–	+(6)	–
Mucor hiemalis Wehmer	–	–	+(1)	+(4)
M. ramonissimus Samutsevitsch	+(480	+(297)	+(9)	+(5)
M. saturninus Hagem	–	–	+(2)	–
** *Paeciliomyces lilacinus* (Thom) Samson	–	–	–	+(1)
** *P. marquandii* (Masse) Hughes	–	–	+(2)	–
** *Penicillium chrysogenum* (Thom) Samson	+(8)	+(9)	+(9)	+(2)
** *P. frequetans* Westling	+(1)	+(1)	+(2)	–
** *P. janthinellum* Biourqe	+(15)	+(17)	+(1)	+(13)
** *P. luteum* Zukal	–	–	+(1)	–
** *P. nigricans* Bain. ex Thom	–	–	+(4)	–
** *P. vermiculatum* Dangeard	–	+(1)	–	+(1)
** *Penicillium* spp.	+(1)	+(1)	+(20)	+(30)
Phoma sp.	–	–	–	+(2)
Scopulariopsis brevicaulis (Sacc.) Bain	+(16)	+(1)	+(1)	–
** *Trichoderma harzianum* Rifai	–	–	–	+(6)
** *T. koningii* Oudem.	–	–	+(3)	–
Trichoderma viride Pers. ex Gray	–	–	+(2)	+(1)
Yeast imperfecti	+(1)	+(1)	–	–
Zygorrhynchus moelleri Vuill.	–	–	+(7)	+(3)

Explanations: * potentially phytopatogenic, ** potentially antagonistic, *** number of isolates.

Table 4

Proportions of potential antagonist and potential phytopathogens in the studied substrates

Substrate	Number of isolates*			
	potentially antagonistic		potentially phytopatogenic	
Arable soil	28		7	
Horticultural substrates	27		8	
Tobacco dust in:				
– arable soil	15[1]	14[2]	28[1]	47[2]
– horticultural substrates	12	16	32	40

Explanations: *Martin's medium: (1) after five weeks; (2) after ten weeks

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Zasiedlanie pyłów tytoniowych przez grzyby

Streszczenie

Pyły tytoniowe (odpad przemysłu tytoniowego) po wprowadzeniu do gleby i podłoża ogrodniczego są szybko zasiedlane przez grzyby obu tych środowisk. Kolonizacja pyłów polega na sukcesji fizjologicznie zróżnicowanych zbiorowisk grzybów. Zbiorowiska te charakteryzują się ubogim składem gatunkowym obejmującym głównie populacje *Mucor ramonissimus, Fusarium solani, F. oxysporum, F. redolens* i *Geotrichum candidum* oraz niekorzystnym pod względem fitosanitarnym układem stosunków mikrobiocenotycznych.