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Microfungi found on phellen of *Jugulans nigra* L. in Southeastern Ohio, USA

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Abstract

Bark surfaces from black walnut (*Jugulans nigra*) trees of three sites in southeastern Ohio, half, five, and ten miles from the Ohio River, were used in late autumn to isolate fungi in culture, as well as to search for the presence of fungal mycelium and spores. The pH of the cork also was determined. Fifty six, mostly cosmopolitan, species of microfungi and several yeasts were isolated, almost all belonging to imperfect form genera, with the largest number of species in the genus *Penicillium*. No relationship of fungal presence to cork pH was found among the sites whose acidity was related to closeness to the industrialized river edge. Relatively few fungi were isolated, and even fewer spores, and only four mycelial growths were found by electron microscopy. Several spores seen could not be matched with fungi that had been isolated. The bark seems to be a trapping surface for spores, rather than a place of fungal growth.

Key words: bark, cork, fly ash spherules, Jugulans, microfungi, phellen

INTRODUCTION

Tree trunk phellen (cork) is a readily available surface upon which fungi may grow. However, the surfaces of tree bark are not obviously covered with fungi or molds. Dickinson (1976) in his survey of fungi on aerial surfaces of higher plants located very few studies of phellen and most of those were related to particular plant pathogens.

In this study, the older cork surfaces of mature *Jugulans nigra* L. (Black Walnut) are examined to determine what micofungi can be isolated from them and what microfungi or spores of them actually can be seen growing on their surfaces by scanning electron microscopy.

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MATERIAL AND METHODS

External pieces of cork were asceptically removed from twelve *Jugulans* tress, four trees in each of three different localities close to the Ohio River in southeastern Ohio (39°N, 82°W) in late autumn. The sites differ in their distances from industralized areas on the river: site A is a half mile from the river; site B is five miles from the river; and site C was ten miles from the river. Chips of cork were placed into sterile petri dishes containing Czapek's agar or malt extract agar, and later some were placed in Sabourard's dextrose agar, V-8 juice (vegetable extract) agar, neopeptone dextrose agar, or cellulose agar to help stimulate sporulation. After several days incubation at 25°C, the plates were examined for fungal colonies which were then isolated into pure cultures for study and identification. Cultures were discarded after 14 days.

The fungi were identified with standard manuals and monographs using

Table 1

Fungi isolated from cork surfaces

Fungus name	Number of times isolated at sites		
	A	B	C
Alternaria humicola Oudemans			2
Alternaria tenuis Nees	6	5	
Aphanocladium album (Preuss) W. Gams		4	1
Ascotricha sp.	1		
Aspergillus aculeatus Iizuka			1
Aspergillus niger van Tieghem	1		1
Botrytis sp.			1
Brachysporium nigrum (Link) Hughes	1	1	
Candida sp.			1
Cladosporium cladosporioides (Fr.) deVries	3	7	4
Epicoccum nigrum Link	2	1	2
Fusarium graminearum Schwabe		3	
Fusarium oxysporum Schlecht.	1	3	1
Fusarium sambucinum Fuckel		1	
Gleosporium sp.	1		
Mucor griseo-lilacinus Povah	2	4	4
Mucor piriformis Fischer	1	2	
Mucor racemosus Fresenius	1		
Mucor varians Povah	2	4	4
Mucor sp.	5		1
Nigrospora oryzae (Berk. et Br.) Petch			1
Penicillium adametzi Zaleski		1	
Penicillium aurantio-candidum Dierckx			1
Penicillium brefeldianum Dodge			1
Penicillium caseicolum Bainier			1
Penicillium commune Thom	1	1	

	A	B	C
Penicillium cyaneo-fulvum Biourge		1	
Penicillium decumbens Thom	1		
Penicillium frequentans Westling	3	4	
Penicillium herquei Bainier et Sartory	1		1
Penicillium implicatum Biourge		1	1
Penicillium jenseni Zaleski		1	
Penicillium lanosum Westling		1	
Penicillium lilacinum Thom		1	
Penicillium lividum Westling	1		
Penicillium multicolor Griogor.-Manoil. et Porad		1	
Penicillium nigricans (Bainier) Thom			2
Penicillium notatum Westling			1
Penicillium oxalicum Currie et Thom		1	
Penicillium paxilli Bainier			1
Penicillium steckii Zaleski			1
Penicillium variabile Westling			1
Pestalotia cocculi Guba	2	2	2
Pestalotia oleandri Guba		1	
Pestalotia stellata Berk. et Curtis		3	3
Phoma prunicola (Opiz) Wollenw. et Hochapf.	1	2	
Pythium sp.	1		
Rhizoctonia sp.	1	3	3
Rhizopus arrhizus Fischer	1		
Stereum pini (Schleich. et Fr.) Fr.	2		
Trichoderma hamatum (Bon.) Bain.	1		
Trichoderma harzianum Rafai	3	5	4
Trichoderma koningii Oud. apud Oud. et Koning	2		2
Trichoderma longibrachiatum Rafai	4	2	4
Trichoderma pseudokoningii Rafai		1	
Tubercularia vulgaris Tode		1	
Yeast species (unidentified)	2		1

light microscopy and cultural characteristics. Other portions of the cork surfaces were air dried, cemented on specimen stubs, and coated with gold for scanning electron microscopic examination. The identified fungi were also examined by scanning electron microscopy for comparative purposes.

Other portions of the cork from each tree were placed into distilled water and homogenized in a blender. The pH of the resulting solution was measured with a pHmeter.

RESULTS

Fifty six different species of microfungi and several unidentified yeasts were isolated in culture media from *Jugulans* cork surfaces (Table 1). Eight species were isolated from trees in all three sites:

PLATE I

Fig. 1. *Pestalotia cocculi conidia from site A isolation.* Line = 10 μm, ×1500. Fig. 2. Possibly *Pestalotia* sp. conidium on bark surface of tree site C. Line = 10 μm, ×1500. Fig. 3. *Aspergillus niger* conidia from site A isolation. Line = 1 μm, ×10000. Fig. 4. Possibly *Aspergillus* or *Penicillium* sp. conidia on bark surface of tree site C. Line = 1 μm, ×10000. Fig. 5. *Penicillium frequentans* conidia from site B isolation. Line = 1 μm, ×10000. Fig. 6. Possibly *Aspergillus* or *Penicillium* sp. conidia on bark surface of tree site A. Line = 1 μm, ×10000

PLATE II

Fig. 7. *Alternaria tenuis* conidia from site B isolation. Line = 5 μm, ×4000. Fig. 8. Possibly *Alternaria* sp. mycelium conidia on bark surface of tree site A. Line = 5 μm, ×6000. Fig. 9. Possibly *Alternaria* sp. mycelium and conidia on bark surface of tree site A. Line = 5 μm, ×6000. Fig. 10. Possibly *Alternaria* sp. mycelium forming conidia on bark surface of tree site B. Line = 5 μm, ×3000. Fig. 11. Possibly *Alternaria* sp. conidium on bark surface of tree site C. Line = 5 μm, ×5000. Fig. 12. *Alternaria tenuis* conidium from site A isolation. Line = 5 μm, ×4000

PLATE III

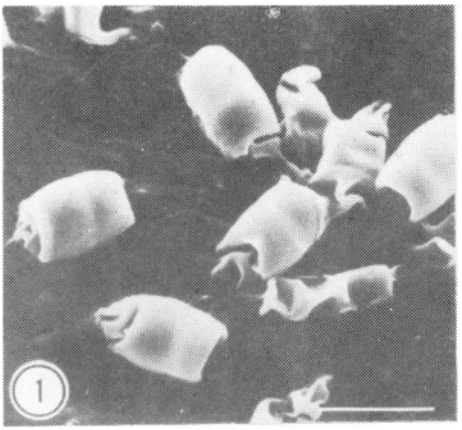
Fig. 13. Fungal hyphae and fly ash spherules on bark surface of tree site A. Line = 1 μm, ×1000. Fig. 14. Possibly fungal mycelium with conidiophores and fly ash spherules on bark surface of tree site A. Line = 10 μm, ×2500. Figs. 15-18. Possibly fungal spores on bark surfaces of trees site C. Line = 5 μm, ×5000

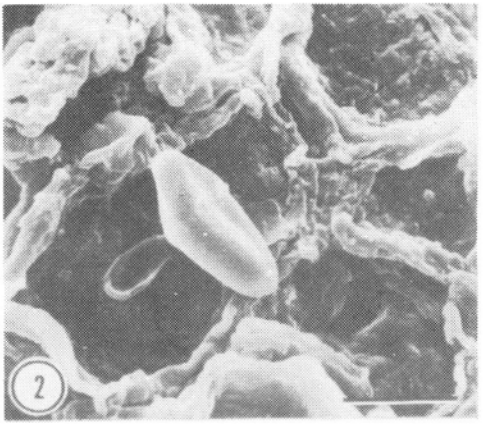
Cladosporium cladosporioides, *Epicoccum nigrum*, *Fusarium oxysporum*, *Mucor varians*, *Pestalotia cocculi*, *Rhizoctonia* sp., *Trichoderma harzianum* and *T. longibrachtiatum*. Five species were isolated more than ten times: *Alternaria tenuis*, *Cladosporium cladosporioides*, *Mucor varians*, *Trichoderma harzianum* and *T. longibrachtiatum*. Most of the fungi were imperfect ones with the largest number of species being found in the genus *Penicillium*. *Stereum pini* was the only basidiomycete found.

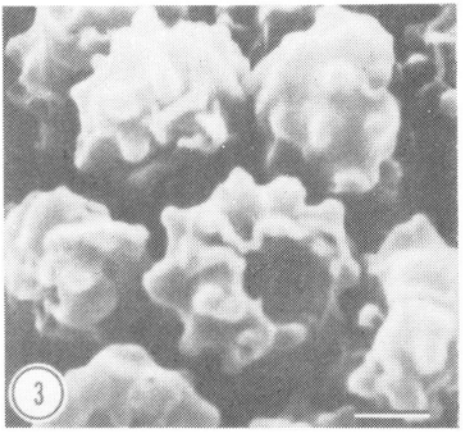
In the scanning electron microscopic examination very few fungi or spores were found. Only four times were mycelium discovered growing on the cork surface. Spores of several types were found and in a few cases it was possible to potentially match them with the spores of the species isolated in culture media. (Figs. 1-12). Spores or mycelium of *Alternaria*, *Penicillium* and *Pestalotia* were identified on the cork and these were among the frequently found genera in the cultural isolations.

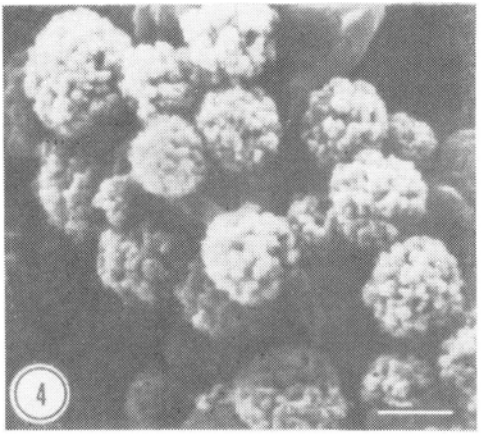
The acidity of the cork was found to be in the pH range of 3.90 to 6.75, with the individual sites having different ranges: site A — 3.90-4.70; site B — 4.90-5.25; and site C — 5.95-6.75. Scanning electron microscopy showed the presence of industrial particles which were particularly abundant on the cork surfaces at site A (Figs. 13, 14).

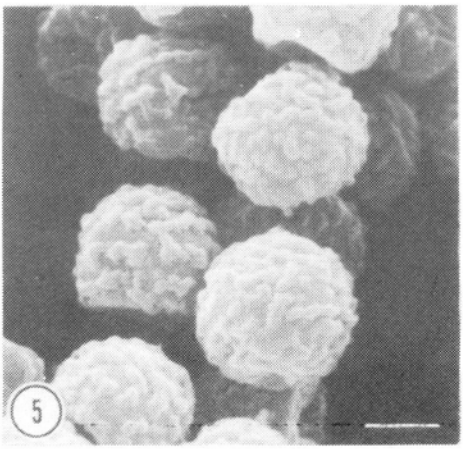
PLATE I

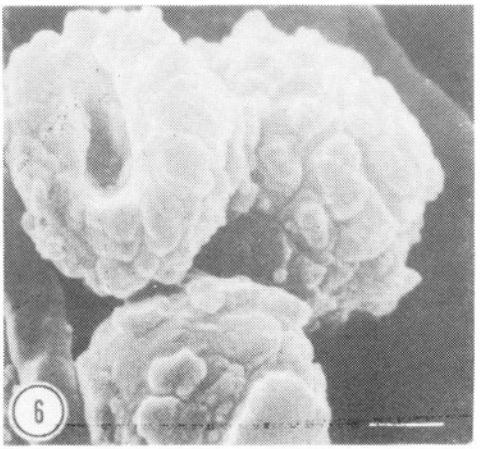












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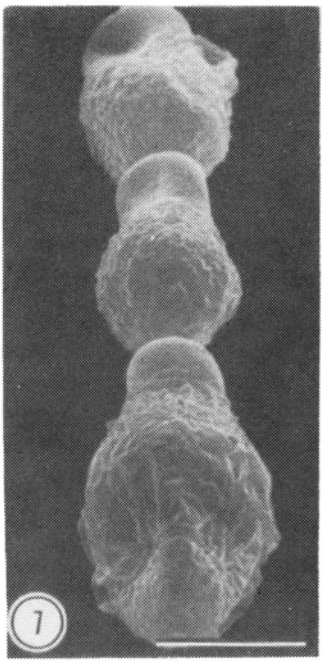

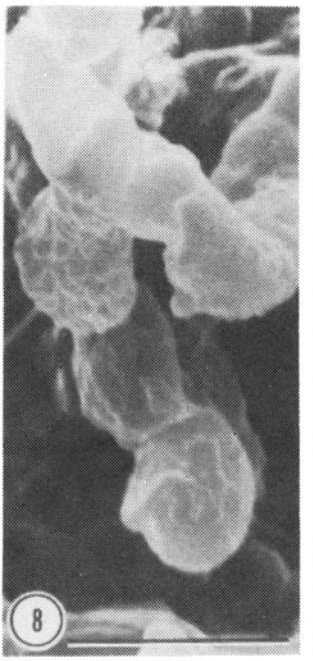

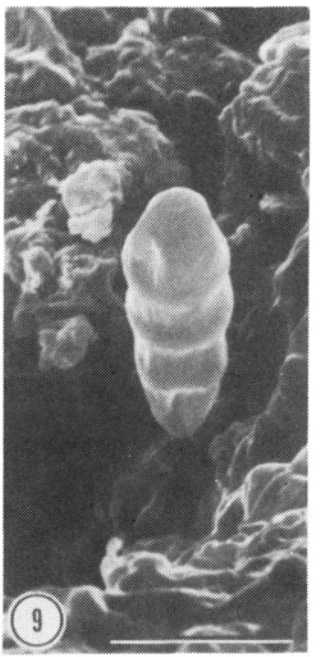

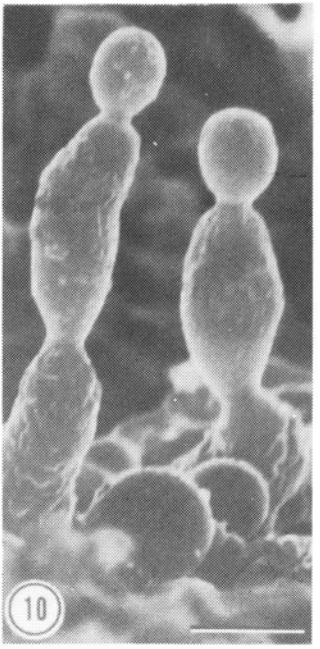

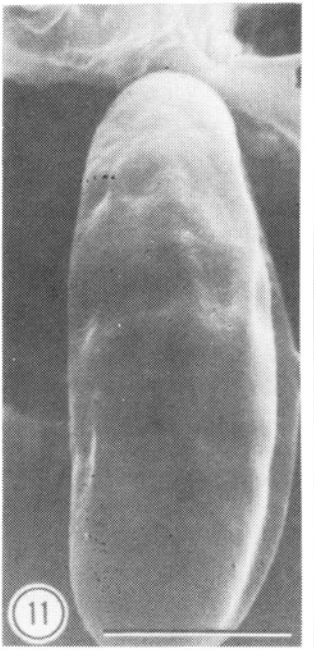

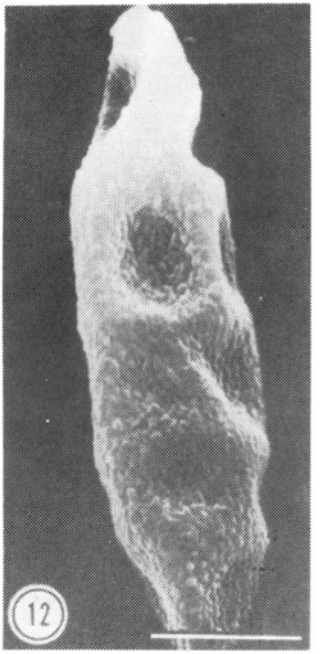

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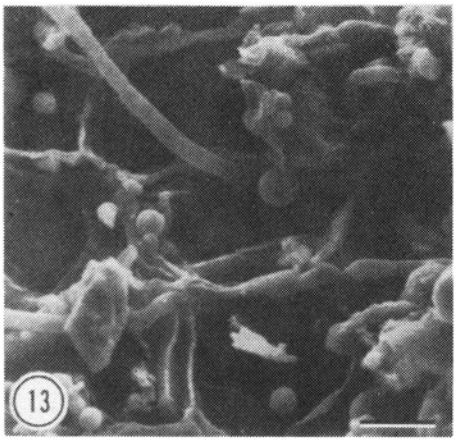

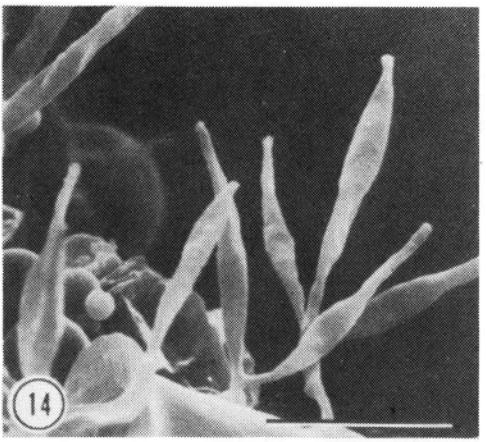

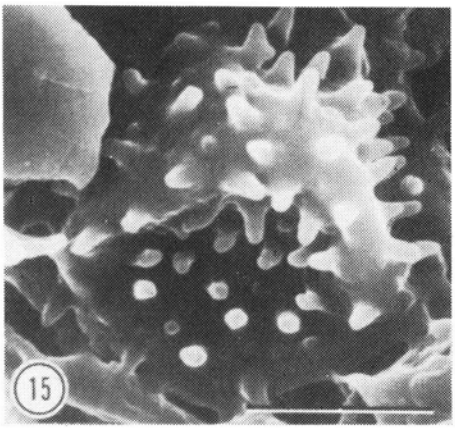

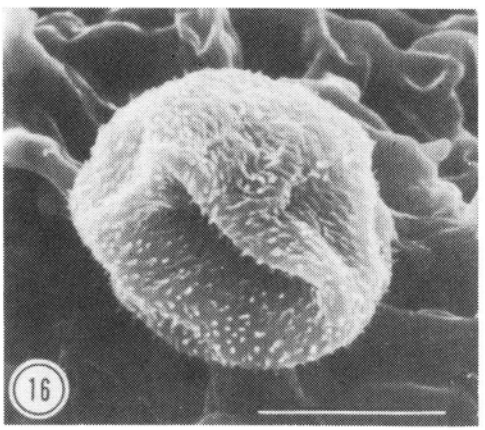

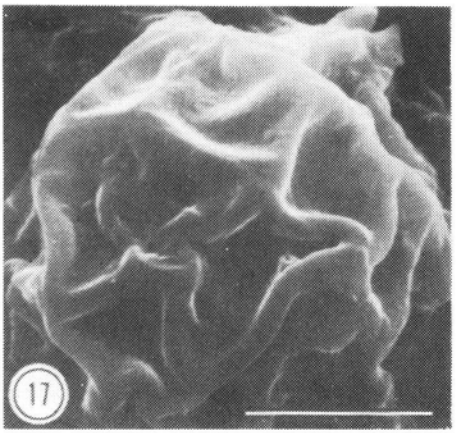

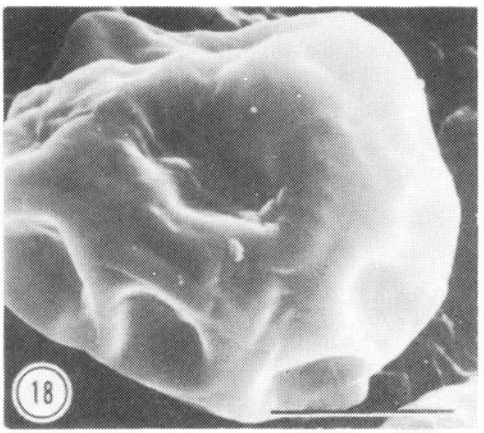

DISCUSSION

The fungi isolated and found are mostly cosmopolitan types, typical of soil (Williams and Schmitthenner 1956, Gilman 1957) and/or are those frequently found by air trapping (Gregory 1973). They are genera that often appear in the few reports of stem surface fungal isolations (Garner and French 1965, Garner 1967, Hudson 1968, Sivak and Person 1973, Updegraff and Grant 1975, Cotter and Blanchard 1982, Melgarejo et al. 1985). Also typical of previous findings are the preponderance of imperfect form genera. Only *Mucor, Pythium* and *Rhizopus* among the lower fungi were found. *Ascotrichia* was the only ascomycete and *Stereum* the only basidiomycete found. The greatest number of species represent those in the genus *Penicillium*. Melgarejo et al. (1985) found *Penicillium* and *Cladosporium* species to be most abundant in their analysis of peach twigs in Spain in late summer, while *Aspergillus* species predominated in autumn. In very wet conditions of late winter and early spring in British Columbia, Sivak and Person (1973) found that 90% of their isolates were *Pullularia* with *Epicoccum*. *Rhizopus* and *Penicillium* making up all of the rest. The Ohio conditions are not as moist as British Columbia nor probably as dry as those of Spain.

The isolation of relatively few fungi in this study indicates that fungal growth and spores is not very common on *Jugulans* cork. Such a conclusion is verified by the scarcity of visible fungal hyphae or spores in examining large areas of surface of cork with scanning electron micoscopy. The surprising aspect of these examinations is that a limited number of those found can be matched with fungi previously isolated in culture. Several spores (Figs. 15-18) are completely different types from the isolates. In only four cases were mycelial growth found (Figs. 8, 10, 3, 14) which makes it likely that most fungi isolated in culture from cork surfaces are not actually growing there, but rather are spores that are simply caught there as were several pollen grains found. Bark surfaces, particularly rough ones, make reasonably good trapping areas for air spora.

The cork pH was typically acidic, however the degree of acidity varied directly with distance from river. The river valley is the site of several power plants and other industrial plants. Several studies have shown that tree bark acidification is an indicator of air pollution, particularly of the presence of SO_2 (Grodzińska 1977, Lotschert and Kohm 1977). Those trees nearest the river (ite A) had numerous fly ash spherules on their cork surfaces (Figs. 13, 14) which shows the direct effect of the industrial presence (Brownlee et al. 1976, Fisher et al. 1976). On the other hand, there is not any correlation between the abundance of fungal isolates and the distance from the river of the trees. Fungi generally grow well in acidic environments.

As a result of this study, there are six findings that can be made:

1. Fifty six different species of fungi occur on the cork surface of *Jugulans nigra* in southeastern Ohio in the autumn.

2. The imperfect fungi are the vast majority of genera found.

3. The number of different isolates was small indicating a limited number of fungi were present.

4. In scanning electron microscopic examination, only four mycelial growths occur, and many of the detached spores did not match those of the isolated species or genera. In general, the surfaces appeared free of fungal growth.

5. The pH of the tree bark surfaces were acidic and the amount varied with distance from the industralized river.

6. The degree of acidity showed no correlation with the amount or type of fungi isolated in culture.

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Grzyby niższe znalezione na korze Jugulans nigra L. w południowo-wschodnim Ohio, USA

Streszczenie

Wczesną jesienią badano grzyby z powierzchni kory drzew czarnego orzecha włoskiego (*Jugulans nigra*), z trzech miejsc w południowo-wschodnim Ohio, odległych 0,5, 5 i 10 mil od rzeki Ohio. Izolowano grzyby *in vitro*, jak też określano obecność ich grzybni i spor na korze. Określano również pH kory drzew. Wyizolowano 56, przeważnie kosmopolitycznych, gatunków grzybów niższych i kilka gatunków drożdży. Większość, były to gatunki form niedoskonałych, z przeważającą liczbą gatunków z rodzaju *Penicillium*. Nie stwierdzono korelacji między obecnością grzybów a pH kory drzew. Natomiast kwasowość kory drzew była uzależniona od bliskości badanego miejsca od uprzemysłowionego brzegu rzeki. Wyizolowano stosunkowo niewiele grzybów i jeszcze mniej spor. Ponadto, przy użyciu mikroskopu elektronowego, znaleziono tylko 4 grzybowe narośle. Wiele znalezionych spor nie mogło być związanych z grzybami, które wyizolowano. Kora wydaje się być raczej powierzchnią wyłapującą spory niż miejscem wzrostu grzybów.