VILNIUS UNIVERSITY
NATURE RESEARCH CENTRE
INSTITUTE OF BOTANY

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RESEARCH ON DISTRIBUTION AND BIOLOGICAL PROPERTIES OF PATHOGENIC FUNGI AND FUNGAL DISEASES OF SMALL-LEAVED LINDEN (*TILIA CORDATA* MILL.) IN VILNIUS CITY

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VILNIAUS UNIVERSITETAS
GAMTOS TYRIMŲ CENTRO
BOTANIKOS INSTITUTAS

Vilma Meškauskienė

MAŽALAPĖS LIEPOS (*TILIA CORDATA* MILL.) PATOGENINIŲ GRYBŲ PAPLITIMO, BIOLOGINIŲ SAVYBIŲ IR JŲ SUKELIAMŲ LIGŲ TYRIMAS VILNIAUS MIESTE

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INTRODUCTION

Green plantings in cities perform protective, aesthetic, recreational and other functions, they also impact human life and climate. Several hundreds of woody plant species are recommended for planting in cities, however, not all assortments is used. In Vinius city, over 100 species of woody plants are being grown; four of them belong to linden (*Tilia* L.) genus: small-leaved linden – *T. cordata* Mill., large-leaved linden – *T. platyphyllos* Scop., European linden – *T. europaea* L., Crimean linden – *T. euchlora* K. Koch., which make up about 80 % of all green plantings (NAVYS, 2005). *T. cordata* is mostly cultivated in city parks, public gardens, communal yards, on tile-covered pavements, green grass; it grows spontaneously all over the Baltic region (NAVASAITIS et al., 2003).

Various abiotic factors in cities (air pollution, salt sprinkle in winter, mechanical injuries, oxygen starvation of roots, etc.) deteriorate the status of trees (HARIS et al., 1999). Weak trees are not resistant to fungal diseases (MONTAGUE et al., 2000; DONALD et al., 2001; SAEBO et al., 2003; BÜHLER, 2006). Fungal diseases decrease ornamental and recreational value of the trees, reduce their age (LONSDALE, 1999; MORTIMER, KANE, 2004).

The investigations of pathogenic fungi on green plantings enable to assess the phytosanitary state of plants and allow us to make a proper selection of trees for urban planting (TIKHOMIROVA, TOBIAS, 1999; KOLEMASOVA, KOVALEVSKAJA, 2000). The most common fungal diseases on urban green plantings are as follows: leaf spot diseases, wood decay and canker caused by the fungi of *Cytospora*, *Nectria* and *Phomopsis* genera (BUTIN, KEHR, 1999; ADAMSKA, BŁASZKOWSKI, 2000; ADAMSKA, 2001; JUHÁSOVÁ, 2002; JUHÁSOVÁ et al., 2003). The fungi of *Cytospora* genera are ascribed to the fungal species prevailing in cities (TOBIAS, TIKHOMIROVA, 2006). These fungi injure over 70 species of woody plants (KEPLEY, JACOBI, 2000; AGRIOS, 2005). The damage caused by fungal disease agents on green plantings is problematic. Until 2005 Lithuanian scientists identified 185 species of fungi on linden trees (*Tilia* L.)

Tilia cordata is very sensitive to environmental changes, therefore, it serves as an indicator for the assessment of urban environment (SNIEŠKIENĖ et al., 1999; ŽEIMAVIČIUS et al., 2003). The analysis of the composition of essential oils of *T. cordata* blossoms collected from different localities enables to reveal the phytosanitary status of linden trees (NIVINSKIENĖ et al., 2007, 2008).

In an urban environment, chemical treatment against disease agents is not applied (or applied only in certain cases), thus, sanitary hygienic and biological measures are used (the Law of Green Plantings by the Ministry of Environment of the Republic of Lithuania (2008)). New anti-microbial biological matters against fungal and bacterial pathogens have been tested. The toxins secreted by yeast as well as many different microorganisms have also been studied, which may be used to localize the infection in plants (MAGLIANI et al., 1997; WEILER et al., 2002; DONINI et al., 2005; MELVYDAS et al., 2006 b).

As far as *Tilia cordata* dominates in Vilnius city, the pathogenic fungi were selected as an object of the research.

The study of fungal disease agents on small-leaved linden in an urban environment is relevant both theoretically and practically due to the assessment of their impact upon plants as well as creation of biological protection measures against linden pathogens.

Pathogenic fungi on *Tilia cordata* in an urban environment were selected as an object of the study, because profound investigations on these pathogens have not been carried out yet.

The aim of the study was to investigate the distribution, biological properties and diseases caused by pathogenic fungi on small-leaved linden (*Tilia cordata* Mill.) in Vilnius city.

The objectives of the investigation were:

1. To investigate the diversity of pathogenic fungi species on small-leaved linden, distribution in different areas of Vilnius city as well as their development peculiarities.
2. To assess fungal disease intensity on small-leaved linden.

3. To analyse the peculiarities of growth *in vitro* of the most harmful fungi on small-leaved linden as well as ascertain their pathogenicity under artificial light in growth chamber and field conditions.
4. To determine the impact of some pathogenic fungi upon chemical composition of essential oils of small-leaved linden blossoms.
5. To study the impact of antagonistic bacteria and *Saccharomyces cerevisiae* killer strains upon fungal disease agents on small-leaved linden as well as estimate the possibilities of their practical application.

Novelty of the work. The diversity of pathogenic fungi species on small-leaved linden in Vilnius city was determined. 21 species of fungi were identified on small-leaved linden for the first time in Lithuania. Two species *Phomopsis irregularis* and *Pseudomassaria chondrospora* are new in Lithuania. A systematic checklist of the recorded fungi species was made. The intensity of fungal diseases was assessed on small-leaved linden. The peculiarities of the most harmful pathogenic fungi growth *in vitro* were investigated and their pathogenicity under artificial light in growth chamber and field conditions was determined. The distribution of compounds of essential oils from small-leaved linden blossoms injured by wide-spread pathogenic fungi *Discula umbrinella*, *Passalora microsora*, *Stigmina compacta* was obtained by chromatographic analysis for the first time. The impact of the identified fungi upon chemical composition of essential oils from small-leaved linden blossoms was estimated. The affect of bacterial isolates T_x and U_x on the species belonging to *Alternaria*, *Cytospora*, *Fusarium* and *Phomopsis* genera was ascertained.

Significance of the study. A systematic checklist of the investigated fungi species on small-leaved linden in Vilnius city was made, which supplements the current data obtained by other investigators. The research on biological characteristics of pathogenic fungi *Cytospora leucosperma* and *Phomopsis velata* extend the knowledge about these fungi in Lithuania. The obtained research data may be included into a large-volumed edition 'Fungi of Lithuania'.

Chemical composition of essential oils from the blossoms of both linden and other plants affected by fungal diseases enables to ascertain the phytosanitary status of plants in an urban environment. Assessment of the impact of bacterial isolates T_x and U_x upon pathogenic fungi *Cytospora leucosperma, Fusarium solani, F. sporotrichioides, Phomopsis velata* on small-leaved linden is important in creating and applying biological control measures. The toxins secreted by yeasts and other microorganisms may be used to localize the pathogene-induced infection in plants.

The research data were introduced in the report 'Observation and recreation of green plantings in Vilnius city', which was presented to the Department of Urban Development of the Municipality of Vilnius city, and in the edition of the Ministry of Environment 'Protection and management of green plantings in an urban environment' (with co-authors).

The statements for defence:

1. In Vilnius city, small-leaved linden are infected with fungi of varying systematic groups, especially with anamorphic fungi.
2. Canker agents *Cytospora leucosperma* and *Phomopsis velata* develop *in vitro* under wide range of media pH and temperature, by light and in the dark; they are pathogenic to small-leaved linden under artificial light in growth chamber and field conditions.
3. Pathogenic fungi *Discula umbrinella, Passalora microsora* and *Stigmina compacta* impact the chemical composition of essential oils from small-leaved linden blossoms.
4. Toxins secreted by bacterial isolates T_x and U_x kill the mycelium of the pathogenic fungi species belonging to *Alternaria*, *Cytospora*, *Fusarium* and *Phomopsis* genera.

Published works. The obtained research data were presented in 6 scientific papers published in reviewed scientific journals, in the material of 6 conferences and the abstracts of 5 conferences. The investigation results were introduced at 4 national and 4 international conferences in Lithuania, 2 international conferences abroad, and 2 symposia.

Volume of the dissertation. The doctoral thesis (in Lithuanian) includes Introduction, Literature Review, Research Material and Methods, Results and Discussion, Conclusions, List of

References (335 citations), List of Publications, List of Abbreviations and 10 Supplements. The dissertation is illustrated with 9 tables, 54 figures. The volume of the dissertation is 158 pages.

RESEARCH MATERIAL AND METHODS

The research material was collected from small-leaved linden trees in Vilnius city in 2005–2007. In total 5113 trees were observed, 530 samples collected and studied.

To isolate pure cultures of fungi, the samples of twigs and leaves were sterilized following the methods by SIEBER et al. (1991). Fungal colonies, which differed according to morphological and cultural features, were resown on malt extract agar (MEA) for further investigations. Macromycetes samples were identified by morphological characteristics of the fruit body.

Fungal species were identified following the manuals for identification of fungal disease agents, monographs and papers by different authors (ARX, 1981; BRAUN, 1999; BRAUN, MELNIK, 1997; ELLIS, 1976, 1997; ELLIS, ELLIS, 1985; GERIACH et al., 1982; GRICIUS, MATELIS, 1996; IGNATAVIČIŪTĖ, TREIGIENĖ, 1998; NELSON et al., 1983; PIRONE, 1978; MELNIK, POPUSHOJ, 1992; MELNIK, 2000; SUTTON, 1975, 1977, 1980; SIMMONS, 2007; URBONAS, 1997, 1999).

Cytospora and *Phomopsis* genera species were investigated by polymerase chain reaction (PCR) method. DNA extraction was performed by using a NucleoSplin®Plant II kit (producer GmbH&Co MACHEREY-NAGEL, Germany), which is based on silica membrane technology.

Identification of fungi DNR sequences was carried out by comparing the obtained sequences with those stored in the Gene Bank database of the National Centre for Biotechnology Information (NCBI) (ALTSCHUL et al., 1997).

Metheorological conditions were evaluated by using the Metheorological bulletin data (SKEIVELIENĖ, 2005, 2006, 2007).

To test the impact of media on micromycetes development as well as to find optimal growth media, the fungi were grown on malt extract agar (MEA), malt extract peptone agar (MEPA), potatoe dextrose agar (PDA), corn meal agar (CMA), Czapek-Dox agar (CDA), Hagem agar (HA).

The impact of hydrogen ions concentration on micromycetes growth was tested on MEA medium, pH 3, 4, 5, 6, 7, 8, 9, 10. The cultures were incubated in a thermostat at +25 °C temperature.

The effect of temperature on growth intensity of colonies was studied by cultivating micromycetes on MEA medium at +5 °C, +10 °C, +15 °C, +20 °C, +30 °C by light and in the dark.

Pathogenicity of micromycetes was investigated by artificial inoculation of small-leaved linden cultivated in growth chamber and field conditions. An artificial inoculation was performed following the technique similar to that described by D. P. WEINGARTNER & E. J. KLOS (1975).

The chemical composition of essential oils was analysed by applying gas chromatography-mass spectrometry methods. Identification of essential oil compounds was based on the comparison of the retention indexes (IR) and the mass spectra with the corresponding data in the literature and computer mass spectra libraries (DAVIES, 1990; ADAMS, 2001).

Killer activity of *Saccharomyces cerevisiae* standard killer strains K7, Rom-K100, M437 and bacterial isolates T_x and U_x (extracted at the Laboratory of Genetics of the Institute of Botany) was determined by their ability to form lysis zones on the lawn of a test α'1 *S. cerevisiae* strain (GULBINIENĖ et al., 2004; SOMERS & BEVAN, 1969; SHERMAN, 1986).

The analysis of variance of fungi species composition on different substrate types was performed using Sǿrensen index (C_s) (SORENSEN, 1948). Frequency of recorded (R_f) fungi species was calculated following CASTILLO et al. (2004). Average damage grade was calculated having modified methodics applied in agriculture and forestry (JUODVALKIS, VASILIAUSKAS, 2002; ŠURKUS, GAURILČIKIENĖ, 2002). Multiple comparisons of treatment means were made by applying confidence interval method ($p<0.05$). Statistical analysis of the data was performed using SPSS 16.0 version and Microsoft® Office Excel 2003 (Statistical Analysis ToolPak) software.

RESULTS AND DISCUSSION

The checklist includes the data on the pathogenic fungi recorded and identified on small-leaved linden in Vilnius city. The taxa of teleomorphic fungi were presented following the system presented in the dictionary of fungi by Ainsworth & Bisby (KIRK et al., 2004). The fungi identified only from their anamorphs, were ascribed to anamorphic fungi group, which is divided into two classes (*Hyphomycetes*, *Coelomycetes*). *Diaporthe eres* Nitsche (anamorph *Phomopsis velata* (Sacc.) Traverso) was identified at anamorph and teleomorph stage. The species of fungi were arranged according to classes in alphabetical order by indicating Latin names, widely used synonyms, authors, anamorphs and teleomorphs. The authors of species names were presented following the data in the website http://www.indexfungorum.org/Names/Names.asp. The substrate on which the fungi had been recorded was also described: the crown of a tree – a twig (diameter <1 cm, on the crown or fallen, living or dead), a branch (diameter ≥ 1cm, on the crown or fallen, living or dead), a dead branch, trunk, roots, stump (broken part of a trunk or that which remained after felling of a tree, not higher than 1 m), living or fallen leaves. The checklist contains fungi substrares and trophic groups (following YU. T. DYAKOV (2007)), the number of localities and their types as well as total frequency of records in Vilnius city (following E. KUTORGA (2000)). (*) Fungi species first time recorded on small-leaved linden. (°) New in Lithuania fungi species.

TAXONOMIC STRUCTURE OF PATHOGENIC FUNGI ON SMALL-LEAVED LINDEN

70 species of pathogenic fungi were recorded on small-leaved linden in Vilnius city in 2005-2007. 7 taxa were described to genus rank. The largest number of species was ascribed to the group of anamorphic fungi. Fungi species belong to 5 classes, 8 orders, 19 families and 63 genera (Table 1).

Table 1. Taxonomic spectrum of pathogenic fungi on small-leaved linden (the number of taxa described to genus rank is indicated in the brackets)

Phylum, group	Number of classes	Number of orders	Number of families	Number of genera	Number of species
Zygomycota	1	1	1	2	2
Ascomycota	1	3	5	5	5(1)
Basidiomycota	1	4	13	19	24
Anamorphic fungi	2	–	–	37	39 (6)
Total number:	5	8	19	63	70

Distribution (%) of fungi taxa on small-leaved linden according to the phyla is indicated in Fig. 1.

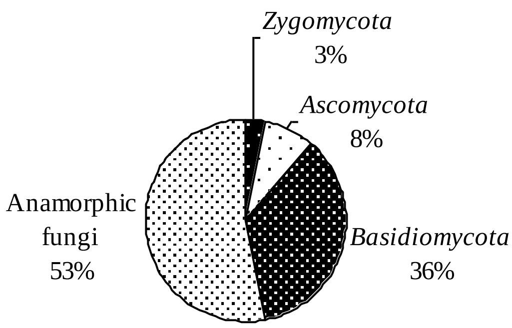


Fig. 1. Distribution (%) of fungi taxa on small-leaved linden according to the phyla in Vilnius city in 2005–2007.

Recently 206 species of fungi have been recorded on linden trees (*Tilia* L.) in Lithuania: until 2005 – 185 species, in the period of research (2005–2007), 21 new species were identified on small-leaved lenden.

SUBSTRATIVE AND TROPHIC STRUCTURE OF FUNGI ON SMALL-LEAVED LINDEN

Substrative structure of fungi. The fungi were recorded on different parts of small-leaved linden: trunks, stumps, roots, living and dead branches (diameter ≥ 1 cm), twigs (diameter < 1 cm), living and fallen leaves (Fig. 2).

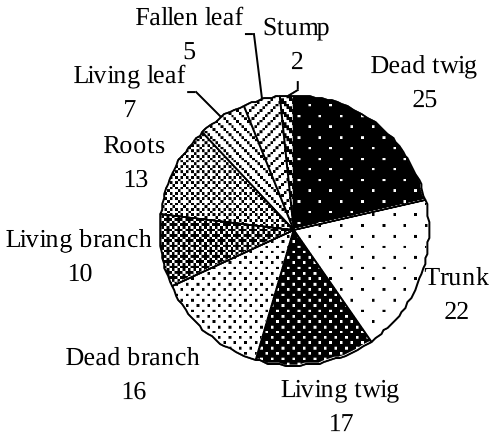


Fig. 2. Distribution of the number of fungi species on small-leaved linden according to substrate types in Vilnius city in 2005–2007.

Most fungi were identified on dead twigs of small-leaved linden (25 species) (Fig. 2). Of these, 2 species were ascribed to *Ascomycota*, whereas 23 species – to the phylum of anamorphic fungi. On this type of substrate we recorded fungi of the *Camarosporium*, *Fusarium*, *Lamproconium*, *Microdiplodia*, *Rabenhorstia*, *Stigmina* (anamorphic fungi), *Hypoxylon*, *Nectria* (*Ascomycota*) genera. *Fusarium solani*, *F. sporotrichioides*, *Nectria cinnabarina*, *Stigmina compacta* were found most frequently on dead twig. A trunk was also reported to be a substrate abundant in fungi species. On this substrate, 22 species belonging to *Basidiomycota* phylum were determined. *Schizophyllum commune* was very common. Fungi of the *Armillaria*, *Bjerkandera*, *Chondrostereum*, *Flammulina*, *Fomitopsis*, *Mycena* genera were occasional, those of the *Ganoderma*, *Inonotus*, *Laetiphorus*, *Oxyporus*, *Phellinus*, *Pholiota*, *Pleurotus*, *Polyporus*, *Stereum*, *Trametes* – very rare. 17 species were detected on living twigs: 1 species belonging to *Ascomycota*, 16 of these – the phylum of anamorphic fungi. Fungi of the *Cytospora*, *Diplodia*, *Fusarium*, *Phomopsis* (anamorphic fungi), *Pseudomassaria* (*Ascomycota*) genera were identified. Fungi of the *Cytospora* genera were the most common on living twigs. 16 species were found on dead branches: *Basidiomycota* – 1 species (*Fomes fomentarius*), *Ascomycota* – 2 (*Chaetomium chartarum*, *Nectria cinnabarina*), the phylum of anamorphic fungi – 13 species, wich are ascribed to the *Alternaria*, *Fusarium* genera. 10 species were detected on living branches: *Ascomycota* – 1 species (*Nectria cinnabarina*), anamorphic fungi – 9 species belonging to the *Alternaria*, *Colletotrichum*, *Fusarium* genera. 13 species were identified on roots: *Zygomycota* - 2 species (*Mucor mucedo*, *Rhizopus stolonifer*), *Ascomycota* – 1 (*Chaetomium chartarum*), the phylum of anamorphic fungi – 10 species belonging to the *Aspergillus*, *Botrytis*, *Cephalosporium*, *Cladosporium*, *Penicillium* genera. On living leaves we detected 7 species belonging to the phylum on anamorphic fungi. Fungi of *Passalora*, *Discula*, *Fumago* genera were the most frequent, whereas those of the *Asteroma*, *Septoria* genera were occasional. 5 species belonging to the *Alternaria*, *Cladosporium* genera were identified on fallen to the ground leaves, 2 species belonging to the *Calocera*, *Phlebia* genera – on stumps.

Comparison of the compositions of fungi species on different types of substrates according to Sørensen similarity index (C_s) allowed us to establish that the most similar compositions were on living and dead branches – C_s = 1.15, living branches and living twigs – C_s = 0.96 as well as dead branches and living twigs – C_s = 0.85. 13–15 common species belonging mostly to the group of anamorphic fungi were identified. Although anamorphic fungi species prevaild on twigs, branches and leaves, however, specific composition of species was characteristic to leaves – C_s = 0.05 – 0.11. All fungi species recorded on a trunk are ascribed to *Basidiomycota* phylum.

Trophic structure of pathogenic fungi. It is rather problematic to strictly define the mode of living of parasytic and saprotrophic fungi. Profound studies on fungal diseases are necessary (SOKOLOVA, 1999 b). The mycobiota of small-leaved lenden was divided into three trophic groups: biotrophs, facultative biotrophs and facultative saprotrophs. Biotrophs attack leaves, branches as well as trunks; they cause different diseases, wood decay.

It was ascertained that in Vilnius city the mycobiota of small-leaved lenden included: obligatory biotroph – 7 species, facultative biotroph – 30, facultative saprotroph – 33 species. 3 species of obligatory biotroph were described to the *Basidiomycota* phylum, 4 species – to the group of anamorphic fungi. All fungi of the *Basidiomycota* phylum were found on the trunks of living trees, whereas anamorphic fungi – on living leaves of the crown. It was established that anamorphic fungi *Passalora microsora* and *Discula umbrinella* were the most characteristic obligatory biotrophs on small-leaved linden. Biotroph *Armillaria mellea* causing tree trunk rot was found on a living trunk of small-leaved linden in Vilnius city.

Facultative biotroph (30 species) were distributed as follows: *Ascomycota* phylum – 2 species, *Basidiomycota* – 10, a group of anamorphic fungi – 19 species. The most characteristic facultative biotroph on small-leaved linden – *Schizophyllum commune* and *Thyrostroma compactum* were recorded correspondingly on a trunk and dead branches.

Facultative saprotrophs (33 species) were ascribed to: *Zygomycota* phylum – 2 species, *Ascomycota* – 3, *Basidiomycota* – 10, a group of anamorphic fungi – 18 species. Facultative saprotrophs belonging to the group of anamorphic fungi were detected on dry twigs and branches, leaves as well as roots. *Fumago vagans* is very common on small-leaved linden leaves. The largest number of fungi species belonging to *Basidiomycota* phylum were found on trunks, less – on stumps, dry branches.

Specificity of substrate, resources and environment are known to be principal factors predetermining the formation of various niches for fungi. Small-leaved linden is not monolithic substrate, therefore, after the comparison of species compositions on leaves, branches, stumps and trunks distinct similarity was not ascertained. This proves that fungi specificaly distribute according to nutrition demand, biological and ecological peculiarities.

DISTRIBUTION AND FREQUENCY OF RECORDED (R_F) SPECIES OF PATHOGENIC FUNGI IN VILNIUS CITY

Distribution of pathogenic fungi. The largest number of fungi species was recorded in Žirmūnai, Kalvarijos, Antakalnis streets, Gediminas av. – 15-32 species, in 14 localities – 5-14 species, 44 localities – 4 and less (Fig. 3).

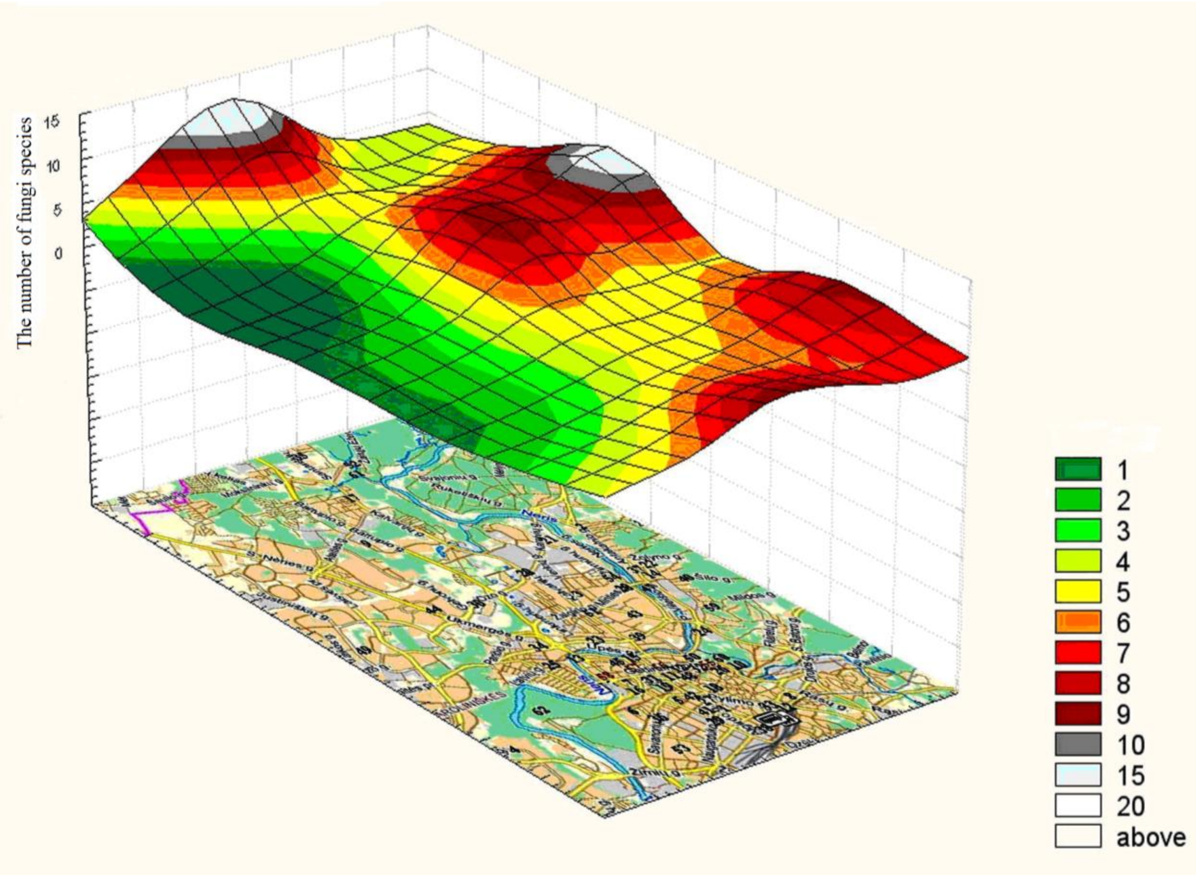


Fig. 3. The number of fungi species on small-leaved linden in different areas of Vilnius city

The highest diversity of fungi species was observed on old trees growing in city parks and some streets. In Verkiai park, 32 species of fungi were identified on small-leaved linden. The diversity of fungi species on green plantings in streets seems to be lower than in parks due to the pruning of branches, gathering of dead leaves as well as other sanitary measures (KOLEMASOVA, KOVALEVSKAJA, 2000).

Basing on Sørensen coefficient values obtained by comparying species composition in different localities, the similarity of localities was determined (Table 2).

Table 2. Sørensen similarity coefficient (C_S) values obtained by comparing fungi species composition on small-leaved linden in different localities. The values in brackets show the same number of fungi species in two localities

	Verkiai park	Žirmūnai st.	Kalvarijos st.	Gediminas av.	Antakalnis st	Naugardukas st.	Sereikiškių park	Pylimo st.	Sapiegos park	Santariškės st.	Kudirkos squere	Klinikos st.
Verkiai park	1											
Žirmūnai st.	0,41(11)	1										
Kalvarijos st.	0,31(8)	**0,63(13)**	1									
Gediminas st.	0,23(5)	0,37(7)	0,11(2)	1								
Antakalnis st.	0,29(7)	0,48(9)	**0,52(9)**	0,45(7)	1							
Naugardukas st.	0,25(6)	0,32(6)	0,47(8)	0,26(4)	0,46(7)	1						
Sereikiškės park	0,17(4)	0,33(6)	0,31(5)	0,27(4)	**0,55(8)**	0,27(4)	1					
Pylimas st.	0,14(3)	0,44(7)	**0,55(8)**	0,23(3)	**0,56(7)**	0,32(4)	0,42(5)	1				
Sapiegos park	0,09(2)	0,18(3)	0,27(4)	0,23(3)	0,32(4)	0,24(3)	0,33(4)	0,31(3)	1			
Santariškės st.	0,24(5)	0,39(6)	0,36(5)	0,24(3)	**0,51(6)**	0,25(3)	035(4)	0,32(3)	0,21(2)	1		
Kudirka squere	0,26(5)	0,27(4)	0.23(3)	0,26(3)	0,27(3)	0,18(2)	0,29(3)	0,24(2)	0,24(2)	0,51(4)	1	
Klinikos st.	0,15(3)	0,27(4)	0,46(6)	0,08(2)	0,45(5)	0,45(5)	0,38(4)	**0,58(5)**	0,24(2)	0,38(3)	0,43(3)	1
Basanavičius st.	0,11(2)	0,36(5)	0,41(5)	0,27(3)	**0,57(6)**	0,38(4)	**0,61(6)**	**0,63(5)**	0,35(3)	0,41(3)	0,46(3)	**0,62(4)**

In the most similar localities, the total number of recorded species came to 6-13. In the localities, where 4-6 species were recorded, the total number of the identified species made up 1-5. In other localities, 1-3 fungi species were found, and the values of Sørensen coefficient were not calculated. Larger number of the same species in the compared localities was conditioned by the larger number of the recorded species. In the localities with a similar number of recorded species, Sørensen coefficient was similar.

Frequency of the recorded species of pathogenic fungi. The records of *Fumago vagans* (40 %), *Passalora microsora* (35 %), *Discula umbrinella* (19 %), *Schyzophyllum commune* (7 %), *Thyrostroma compactum* (6 %), *Cytospora leucosperma* (3 %) were the most frequent in Vilnius city in 2005-2007.

Micromycetes infect trees and cause globally common fungal diseases – leaf spot and canker. 11 species of leaf spot disease agents were identified on small-leaved linden Vilnius city. Leaf spot disease agents *Discula umbrinella, Fumago vagans* and *Passalora microsora* were frequent on living leaves. Other species *Asteroma tiliae* and *Septoria tiliae* identified on living leaves were ascribed to very rare, *Alternaria alternata* was frequent on leaves, *Cladosporium cladosporioides* – quite rare.

Spot disease agents on small-leaved linden leaves are dramatically widespread in different countries (CASTAÑEDA, BRAUN, 1989; CAETANO-ANOLLES et al., 2001). The data obtained by the investigators from other countries confirm rather frequent distribution of leaf spot disease agents: in Estonia – 3 species, Poland – 3, Russia – 11, Slovakia – 2, Great Britain – 7, Germany – 3, Romania – 1, Austria – 1 (PHILLIPS, BURDEKIN, 1982; BUTIN, KEHR, 1999; KOLEMASOVA, KOVALEVSKAJA, 2000; JUHÁSOVÁ et al., 2004; RUSZKIEWICZ-MICHALSKA, 2006; KALAMEES, SAAR, 2006).

Fungus *Lamproconium desmazierii*, which was detected on twigs and smaller branches of small-leaved linden in Vilnius city is known to be recorded in all Europe (PHILLIPS, BURDEKIN, 1982).

Widely distributed fungi of *Cytospora* genus infect small branches of small-leaved linden by causing cytospora canker. Cytospora canker is widely distributed on different plants in natural and urban environment all over Europe, North and South America, Asia and Russia (GVRITIŠVILI, 1982). *Cytospora leucosperma* Sacc. was detected on small-leaved linden in Vilnius city. This species is very common on *Tilia* L. and *Populus* L. in Saint Petersburg (TOBIAS, TIKHOMIROVA, 2006). In Russia and Poland, *Cytospora carphosperma* Fr. was identified (WEBER-CZERWIŃSKA, 1974; SOKOLOVA, 1999a).

Phomopsis cankes agents *Phomopsis irregularis* (Died.) Petr. and *P. velata* (Sacc.) Traverso were detected on small-leaved linden branches in Vilnius city. *P. irregularis* was detected for the first time in Lithuania. *P. velata* was detected on small-leaved linden in Moscow (SOKOLOVA, 1999b).

Most of the macromycetes detected on linden trees in Vilnius city are described as capable to parasitize on other species of trees in various countries. *Nectria cinnabarina* was also frequently detected on small-leaved linden branches. Fungi of the *Nectria* genus cause canker on branches of *Acer*, *Fagus*, *Fraxinus*, *Platanus* and *Tilia* genera (SOKOLOVA, 1999b; TELLO et al., 2005). In Estonia, 7 species of fungi were recorded on small-leaved linden: *Thanatephorus fusisporus*, *Tomentella stuposa*, *Mycena haematopus* var. *haematopus*, *M. galericulata*, *Laetiporus sulphureus*, *Pleurotus pulmonarius*, *P. ostreatus* (BRESINSKY, 2006; KALAMEES, SAAR, 2006). Of these, the latter 4 fungi species were recorded on small-leaved linden in Vilnius city. Fungi *Phellinus igniarius*, *Bjerkandera adusta*, *Polyporus varius* detected in Estonia on *Betula* sp., *Salix* sp. were also recorded on small-leaved linden in Vilnius city. In Finland, the research on fungi causing wood decay proved *Ganoderma applanatum* (Pers.) Pat. G.F. Atk. (=*Ganoderma lipsiense* (Batsch)) to be a particularly harmful macromycete to *Tilia* L. and *Acer* L. trees (TERHO et al., 2007). Fungus *Ganoderma applanatum* was detected on trunk of small-leaved linden in Vilnius city.Parasitic fungus

Armillaria mellea was also identified on a trunk, which is known to be the most harmful pathogene in the forests of Southern and Northern Europe (TELLO et al., 2005).

Most fungi were not specific of small-leaved linden. Typical species were as follows *Fumago vagans* (R_f 40 %) and *Passalora microsora* (R_f 35 %). However, the later fungi and biotroph *Discula umbrinella* (R_f 19 %) were also detected on other species of linden. *Discula umbrinella* was also identified on the leaves of *Aesculus* L., *Betula* L., *Fagus* L., *Fraxinus* L., *Quercus* L. (HAMMERLI et al., 1992).

Schizophyllum commune (R_f 7 %) was frequent not only on a trunk of small-leaved linden, but also on the trees of other genera. *Lamproconium desmazieresii* and *Septoria tiliae* (R_f 0,02 %) very rare on small-leaved linden, but they can also be detected on other species of linden. *Cytospora leucosperma* (R_f 0,8 %) causing cytospora canker on branches of different species of linden (*Tilia* L.) was detected on 115 species of plants (GVRITIŠVILI, 1982).

Under high frequency of recorded pathogenic species, fungous disease may reveal itself weakly (single spots on leaves, only some dry twigs, etc.) and, on the contrary, under low frequency of records, the fungus sometimes may cause epiphytoty (KOLEMASOVA, KOVALEVSKAJA, 2000). All species detected on small-leaved linden in Vilnius city were divided into groups according to total frequency of records: 8 – frequent, 3 – quite frequent, 4 – quite rare, 9 – rare, 46 – very rare.

FUNGAL DISEASE INTENSITY ON SMALL-LEAVED LINDEN

Small-leaved linden disease intensity varied in different habitats. On green plantings of streets, the highest damage grade (1–4) was indicated. In squares, parks, public gardens disease intensity reached 1–2 grades. The highest disease intensity – 4 grades was determined in one street, 3 grades – 18 streets, 3 parks, 1 square, 2 grades – 50 streets, 1 park, 3 squares, 1 grade – 37 streets, 3 parks, 4 squares. The trees were damaged mostly by *Cytospora leucosperma*, *Discula umbrinella*, *Fumago vagans*, *Passalora microsora*.

The highest intensity of disease was ascertained in Ozas street, where average grade of damage of small-leaved linden made up 3.35 grades. In this street, linden trees were infected mostly with *Cytospora leucosperma*.

Leaf spot disease agent *Passalora microsora* damaged small-leaved linden in 38 streets, 3 squares, 4 parks. Pathogene-induced infection was the most intensive in 3 parks, 2 streets, where average grade of damage reached 2.3–3 grades.

Leaf spot disease agent *Discula umbrinella* infected small-leaved linden in 31 streets. The infection was most intensive in 8 streets, where average grade of damage reached 2–2.5 grades.

Sooty mould agent *Fumago vagans* was detected in 32 streets of Vilnius city. Average grade of small-leaved linden damage in 6 streets was 2.5–3 grades, in 12 streets – 2–2.5, in 14 streets – 1–1.5 grades.

Thyrostroma canker agent *Thyrostroma compactum* caused damage in 13 streets, *Schizophyllum commune* – 19 streets. The above-mentioned disease agents were recorded on the same trees as spot disease agents, however, they damaged less number of trees than leaf spot disease agents and their damage intensity was lower – about 1–2 grades.

Average grade of damage in all investigated localities in 2005–2007 varied insignificantly (Fig. 4). Only in Ozas street, intensive distribution of cytospora canker was observed in 2005–2006. The damage reached on average 3.35 grades. After cutting of badly damaged linden trees, in summer of 2006 the average grade of damage was 2.78.

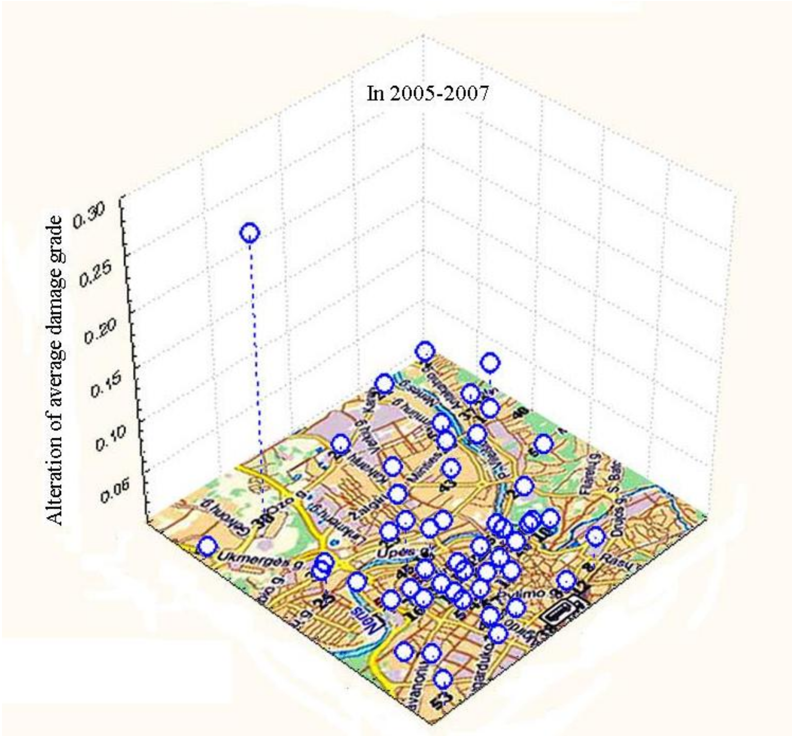


Fig. 4. Alteration of the average damage grade of small-leaved linden on Vilnius city, 2005–2007.

ASSESSMENT OF TAXONOMIC RELATIONSHIP BY POLYMERASE CHAIN REACTION (PCR) METHOD

It is difficult to identify fungi of some genera to species level according to host plant, morphological or physiological characterization. Molecular methods are known to be used to specify species determination.

Cytospora and *Phomopsis* genera species detected on small-leaved linden in Vilnius city were investigated by polymerase chain reaction method.

10 *Phomopsis* and 9 *Cytospora* isolates were investigated by using ITS4 and ITS5 primers for DNA amplification and sequence analysis. By polymerase chain reaction (PCR) method were amplified the fragments of 600 to 650 bp in size (Fig. 5).

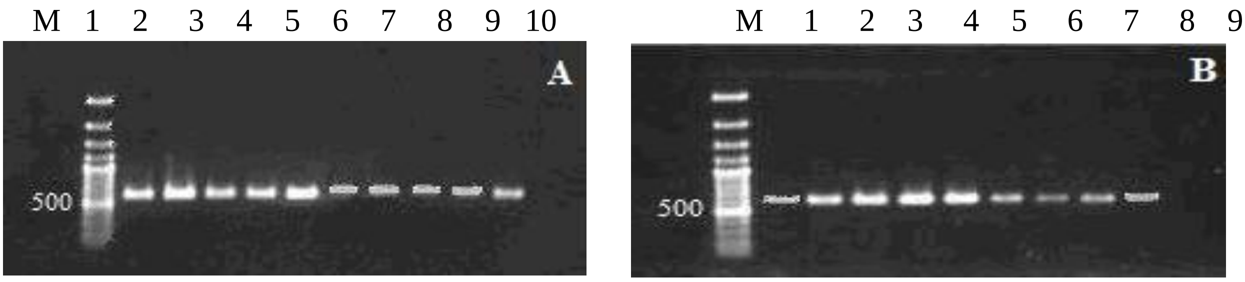


Fig. 5. PCR amplification products by using ITS4 and ITS5 primers. **A**: M – marker, 1–10 *Phomopsis* isolates (P01, P02, P03, P07, P14, P16, P35, P37, P39, P40); **B**: M – marker, 1–9 *Cytospora* isolates (C05, C06, C13, C17, C29, C30, C31, C32, C33)

DNA sequences were compared with those stored in the Gene Bank BLAST database of the National Centre for Biotechnology Information.

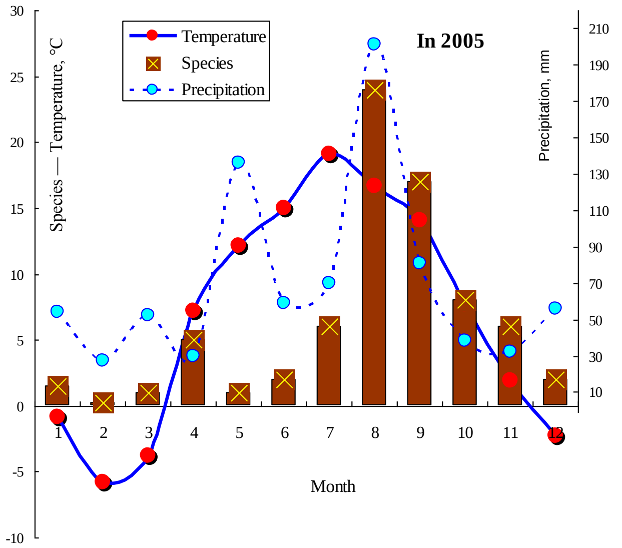
It was established that DNA sequences of *Cytospora* isolates C04, C13 and C17 tally by 100 % with the sequences of fungus *Cytospora rhodophila* Sacc. (number DQ 243809) in BLAST database. In other countries, it has also been reported on common dog rose (*Rosa canina* L.) (SOLOMACHINA et al., 1998; ADAMS et al., 2006). *Cytospora rhodophila* is known to have 20 synonyms and the taxa are not clear. G. ADAMS and other investigators (2006) of *Cytospora* genera fungi maintained that *C. rhodophila* belongs to the group of fungi, which includes taxa ascribed to *C. leucosperma* species. To describe the *Cytospora* fungi species recorded on different plants, it is necessary to distinguish, which synonyms should be attributed to *C. leucosperma* species, and which – not.

All DNA sequences of *Phomopsis* isolates P01, P02, P03, P07, P14, P35, P40 are identical. *Phomopsis velata* DNR sequences are not stored in the Gene Bank BLAST database, therefore, it is not possible to compare with DNA sequences obtained by us. *Phomopsis welata* was identified by morphological features and basing on literature references.

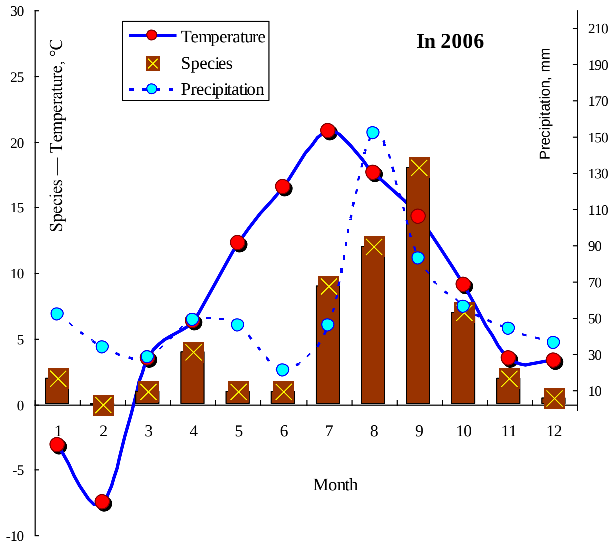
IMPACT OF ECOLOGICAL CONDITIONS UPON THE DISTRIBUTION OF FUNGI ON SMALL-LEAVED LINDEN

Metheorological conditions. Development of fungi species depend upon substrate characteristics, metheorological conditions and other factors. Formation of fungi fruit bodies is known to depend upon temperature and precipitation amount (ARNOLDS, 1981).

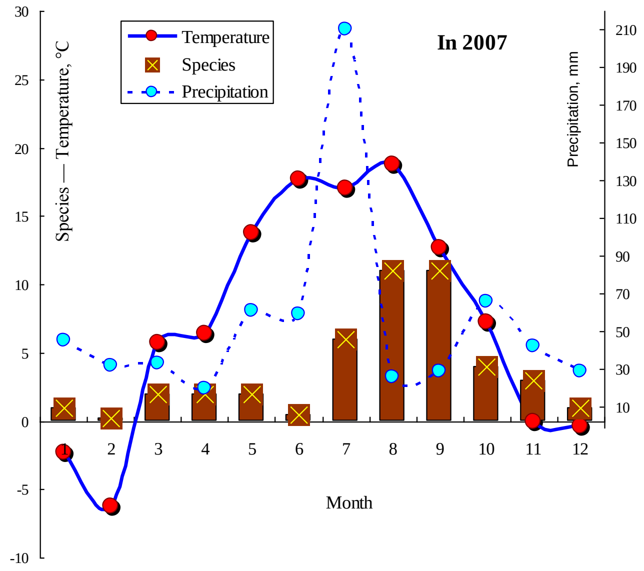
In the research period, the development of pathogenic fungi on small-leaved linden was influenced by temperature and precipitation amount: under high average air temperature and large amount of precipitation, the largest number of fungi species was recorded (Fig. 6).



A



B



C

Fig. 6. Comparison of the number of fungi species recorded on small-leaved linden with the average air temperature and precipitation amount: **A** – in 2005, **B** – in 2006, **C** – in 2007.

The largest number of fungi was detected in August (12–24 species) and September (11–18 species). In April (2–5 species), July (6–9), October (4–8), November (3–6) smaller numbers were reported. The least numbers of fungi were established in March (1-2 species), May (1–2), June (2–3 species). In December and January of the discussed period, the weather was unusually warm, therefore, only 1–2 species of fungi were detected. In February fungi species were not determined.

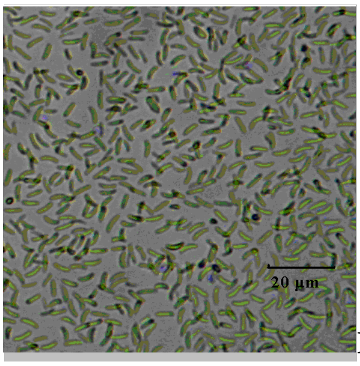
Development of fungal disease agents is more intensive, when average air temperature is about +15–30 °C and mean precipitation amount – 160–200 mm. The largest number of fungi species was determined in August-September of 2005–2007.

RESEARCH ON BIOLOGICAL CHARACTERISTICS OF *CYTOSPORA LEUCOSPERMA AND PHOMOPSIS VELATA*

Fungi of the *Cytospora* and *Phomopsis* genera cause canker diseases on different trees and shrubs. On green planting in cities, most often the *Cytospora* fungi – saprotrophs dominate, which are detected on dead branches of different trees and shrubs, but some species under certain conditions may occur on weak but still living plants and hasten their drying, for example, fungus *Cytospora leucosperma* recorded on living linden trees (SOKOLOVA, 1999a). *Cytospora leucosperma* is ascribed to the group of dominating species on green plantings in cities (TOBIAS, TIKHOMIROVA, 2006). In Vilnius city, *Cytospora leucosperma* was detected on living branches of small-leaved linden and secreted into a pure culture (Fig. 7). In some habitats, on the same branches with the *Cytospora* fungi, the agent *Phomopsis velata* was recorded (Fig. 8). This fungus is rarely detected, but harmful to linden trees, too.

Cytospora leucosperma and *Phomopsis velata* fungi seldom occur alone in their natural habitat without other microorganisms. If we wish to work with them they have to be separated from other microorganisms. It is sometimes possible to obtain a pure culture by directly inoculating a suitable medium. So far as there are no research data on pure *Cytospora* *leucosperma* and *Phomopsis velata* cultures, isolated from the damaged branches of small-leaved linden, therefore, the peculiarities of the fungi development on different substrates under varying environment regime conditions were studied and optimal nutrition media for a particular fungus species were selected. Knowing of their biological features supplement the knowledge on pathogene development possibilities in natural environment, and is useful in pathogenicity studies as well as description of fungi and is essential for successful control of fungal diseases.


A



B

Fig 7. **A** – C*ytospora leucosperma*: *C. leucosperma* pure culture on MEA, **B** – conidia (×400)


A

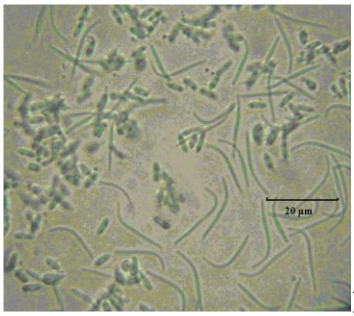
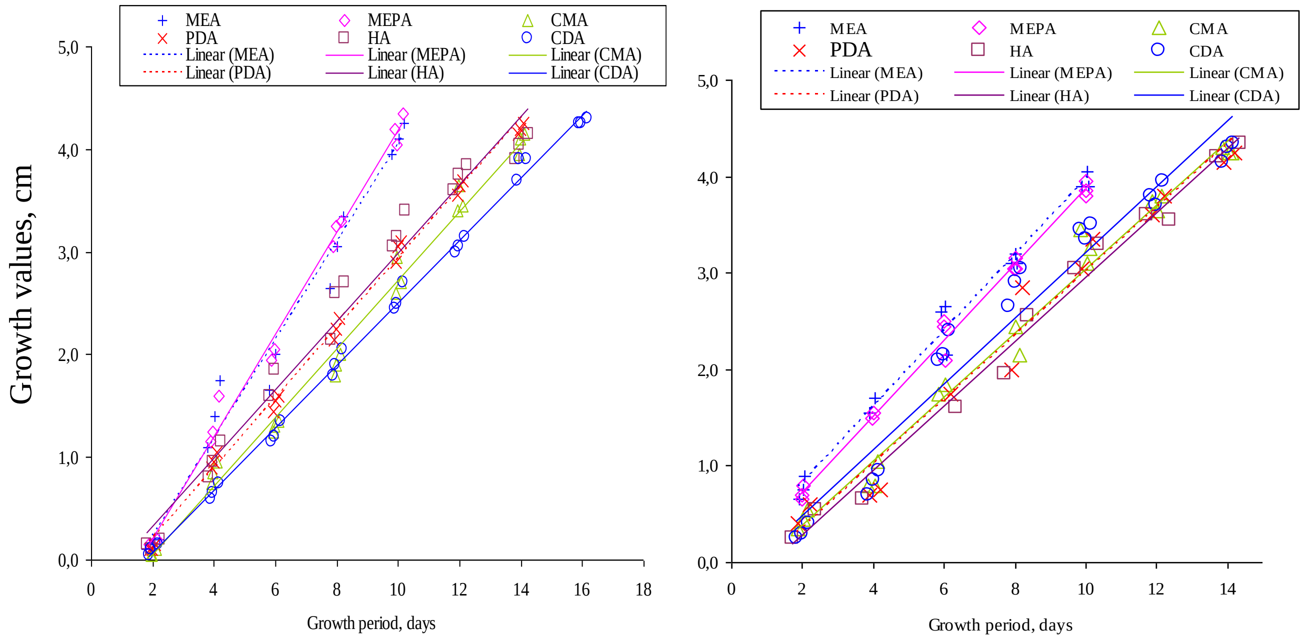

B

Fig 8. **A** – *Phomopsis velata*: *P. velata* pure culture on MEA, **B** – α and β conidia (×400)

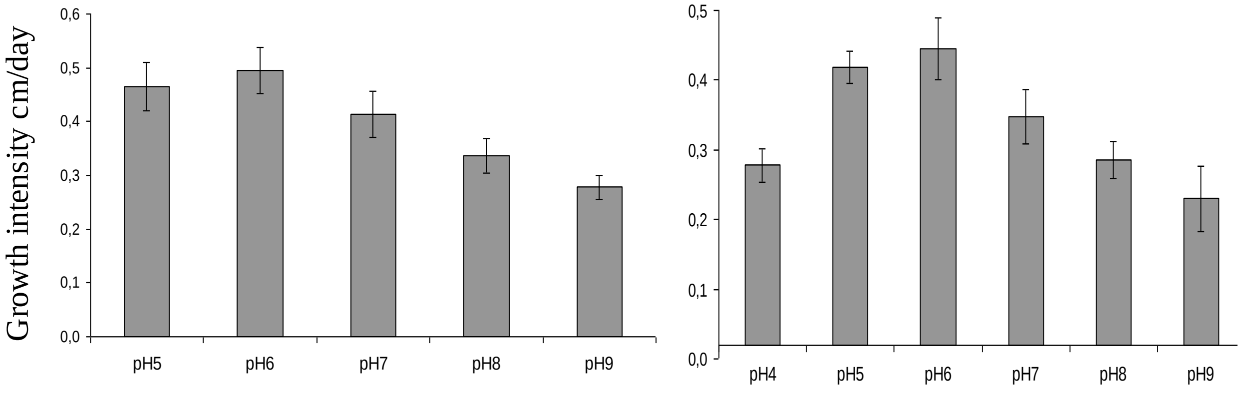
***Cytospora leucosperma* and *Phomopsis velata* development on different nutrition media.** The experiment revealed that *Cytospora leucosperma* and *Phomopsis velata* fungal colonies grew and sporified very well on MEA, MEPA media, worse – on HA, CMA, PDA, and the worst – on CDA medium (Fig. 9, A, B).



A **B**

Fig. 9. Growth values of *Cytospora leucosperma* (**A**) and *Phomopsis velata* (**B**) colonies on MEA, MEPA, HA, CMA, PDA and CDA media.

Impact of pH on the growth of *Cytospora leucosperma* and *Phomopsis velata* colonies. Fungal colonies were growing very well, when pH of the medium was 5-8, moderately – pH 9 (Fig. 10). Whereas at pH 3, 4 and 10 – the pathogen was not growing at all.

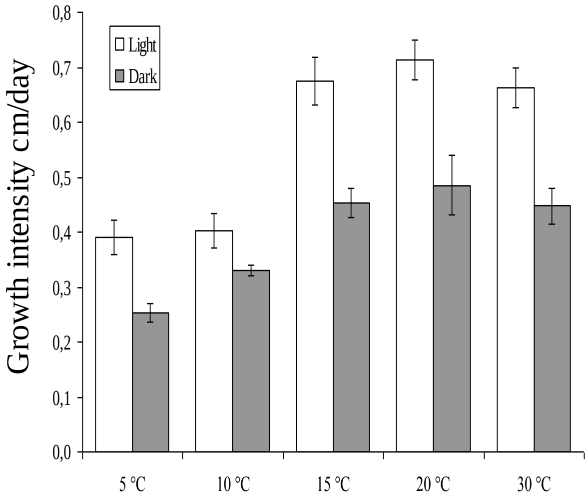


A **B**

Fig. 10. Average growth values of *Cytospora leucosperma* (**A**) and *Phomopsis velata* (**B**) colonies, pH 4-9

Cytospora leucosperma and *Phomopsis velata* are resistant to pH fluctuations. They can grow within wide pH ranges, but do not grow at pH 3 or pH 10.

Impact of temperature and light regime on the growth of *Cytospora leucosperma* and *Phomopsis velata* colonies. By cultivating *Cytospora leucosperma* and *Phomopsis velata* in the light, we determined that growth intensity increased from +15 °C to +30 °C and after 8 days the fungi formed conidiomata. At lower temperature, i. e. +10 °C and less, growth intensity decreased. The growth was also less intensive when the temperature exceeded +30 °C, whereas at +35 °C – fungi were not growing (Fig. 11).



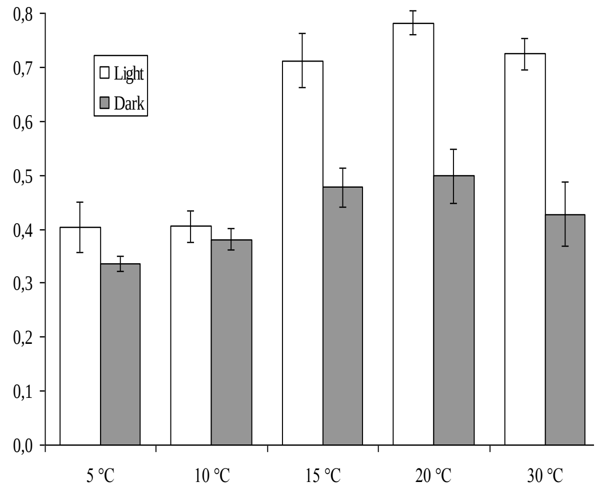


Fig. 11. Average increase of the diameter of *Cytospora leucosperma* and *Phomopsis velata* colonies by cultivating in the light and in the dark at +5, +10, +15, +20 and +30 °C.

The obtained data demonstrated that MEA and MEPA media ensure optimal growth of *Cytospora leucosperma* and *Phomopsis velata* pathogens. The above-discussed media were used for more profound studies of these fungi. Both fungal species may grow within a wide range of hydrogen ion concentration, at pH 5–8. The impact of temperature upon *C. leucosperma* and *P. velata* growth both in the light and in the dark was similar, however, in the dark the intensity of growth was lower, optimal temperature reached between +15 °C and +30 °C. Since the fungi may develop within a wide range of pH and temperatures, it is possible to carry out pathogenicity studies *in vitro*, where the temperature and pH of the substrate cannot be regulated.

PATHOGENICITY OF *CYTOSPORA LEUCOSPERMA* AND *PHOMOPSIS VELATA* TO SMALL-LEAVED LINDEN

Pathogenicity of *Cytospora leucosperma* and *Phomopsis velata* was investigated by using artificial inoculation of small-leaved linden cultivated in growth chamber and field conditions. The pathogenicity was assessed by the increase of the length of necrotic wounds as well as by reisolation of the culture and comparison with the studied culture.

Necrosis of small-leaved linden inoculated with *Cytospora leucosperma* and *Phomopsis velata* both in the growth chamber and field conditions most intensively developed during the first 14 days after inoculation, and later from 14 to 42 days it was spreading insignificantly (Table 3)

Table 3. Average increase of wound length after inoculation of small-leaved linden with *Cytospora leucosperma* and *Phomopsis velata* in growth chamber and field conditions

Number of days after inoculation	Average increase of wound lenght (mm)			
	In field conditions		In growth chamber	
	Cytospora leucosperma	*Phomopsis velata*	*Cytospora leucosperma*	*Phomopsis velata*
14	14,5	16,5	12,4	14,3
28	4,1	5,2	4,9	4,2
42	4,7	3,2	3,9	4,1

After inoculation of linden with *Cytospora leucosperma* in growth chamber, determination coefficient was $R^2 = 0.9997$, whereas after inoculation in field conditions – $R^2 = 0.9976$ (Fig. 12). When linden were inoculated with *Phomopsis velata* in growth chamber, determination coefficient was $R^2 = 0.9989$, whereas after inoculation in field conditions – $R^2 = 0.9999$ (Fig. 13). Determination coefficient revealed that in all research cases the length of wound depended (99 %) on the interval of time after inoculation.

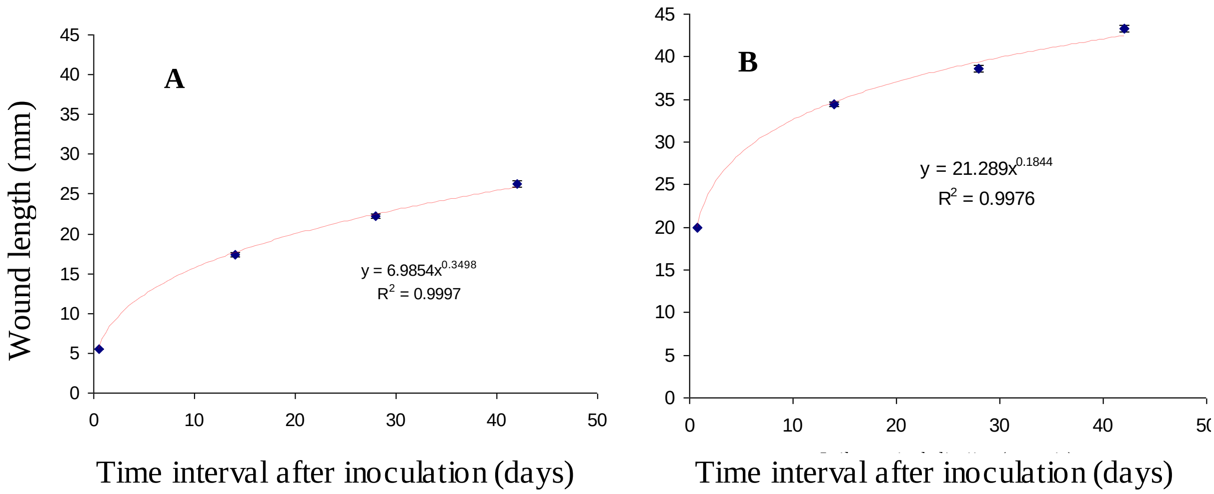


Fig. 12. Small-leaved linden wound length variation (mm) 14, 28 and 42 days after inoculation with *Cytospora leucosperma*: A – in growth chamber, B – in field conditions

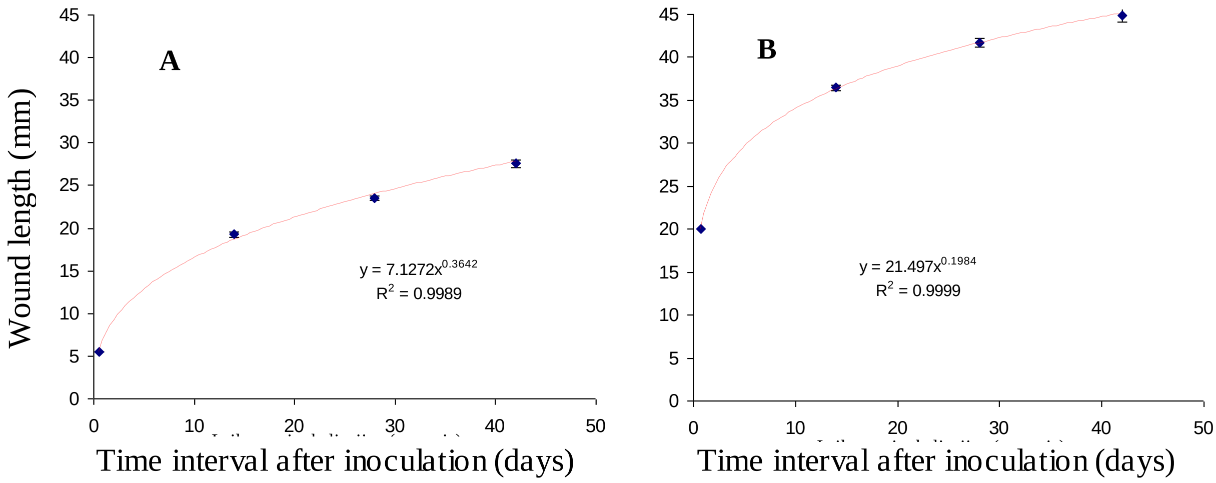


Fig. 13. Small-leaved linden wound length variation (mm) 14, 28 and 42 days after inoculation with *Phomopsis velata*: A – in growth chamber, B – in field conditions

During the first 14 days after inoculation with *Phomopsis velata*, necrotic wounds were increasing faster than those inoculated with *Cytospora leucosperma*. Later the development of both fungi was similar. On control linden trees infected only with malt extract agar (MEA) without fungus mycelium, the length of wound did not change (in growth chamber it was 5 mm, whereas in field conditions – 20 mm). After inoculation of healthy small-leaved linden with *Cytospora leucosperma* and *Phomopsis velata*, distinct differences in necrotic wound size were not observed neither in growth chamber nor under field conditions. Fungus mycelium under field conditions and in growth chamber was developing similarly.

Pure fungi cultures *in vitro* were cultivated on inoculated twigs after 19 months, which confirmed that necrosis was caused by *Cytospora leucosperma* and *Phomopsis velata*. The studies on fungi pathogenicity are known to be conducted (HELTON, 1962; GVRITIŠVILI, 1982; OLD et al., 1986), however, the pathogenicity of *Cytospora leucosperma* and *Phomopsis velata* to small-leaved linden has not yet been investigated. The research proved and confirmed the pathogenicity *Cytospora leucosperma* and *Phomopsis velata*. The first symptoms of bark necrosis manifested 14 days after inoculation of healthy small-leaved linden trees with the above-mentioned fungi in growth chamber under artificial light and field conditions.

INVESTIGATION ON THE CHEMICAL COMPOSITION OF ESSENTIAL OILS OF SMALL-LEAVED LINDEN BLOSSOMS

Chemical composition of essential oils of linden blossoms in natural environment has already been studied (BUCHBAUER G. et al., 1995; PRĄCZKO A., GORA J., 2001). More than 100 different compounds have been identified. However, the essential oils of small-leaved linden growing in urban environment and affected by fungal pathogens have not yet been investigated. Essential oils are known to be applied not only in pharmacology, but also for plant protection from diseases.

The flowers from small-leaved linden trees were collected in urban habitats in 2006–2007: from healthy trees and those damaged by *Discula umbrinella*, *Passalora microsora* and *Stigmina compacta*.

Chromatographical analysis of essential oils of small-leaved linden blossoms collected from both healthy and affected by fungal pathogens trees revealed that in 2006 there were 169, whereas in 2007 – 164 compounds.

The identified compounds were grouped into classes. Mean values of the obtained data are presented in the diagram (Fig. 14).

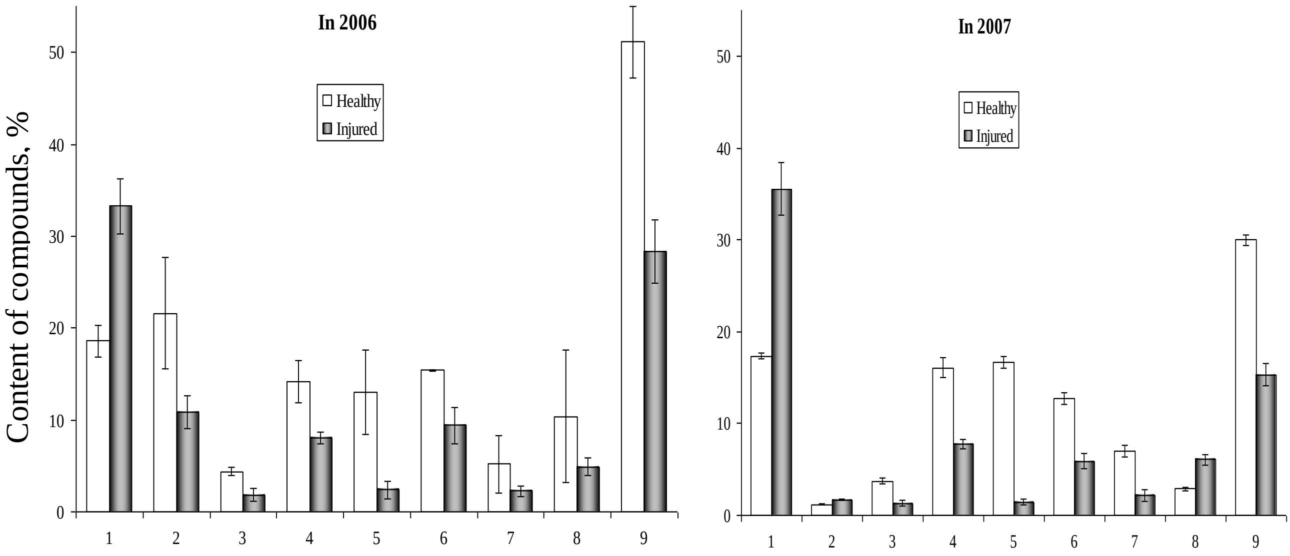


Fig. 14. Diagram of mean distribution (%) of the compounds essential oils of healthy small-leaved linden blossoms and injured by fungal pathogens: 1 – aliphatic hydrocarbons, 2 – oxygenated aliphatics, 3 – monoterpene hydrocarbons, 4 – oxygenated monoterpenes, 5 – sesquiterpene hydrocarbons, 6 – oxygenated sesquiterpenes, 7 – oxygenated aromatics, 8 – aliphatic acids, 9 – oxygenated hydrocarbons

In 2006 and 2007, the content of compounds identified in the essential oils of small-leaved linden blossoms varied insignificantly. Aliphatic hydrocarbons dominated in the affected linden blossoms (in 2006 – 35.6 %, in 2007 – from 39.7 % to 40.7 %). The amounts of monoterpene hydrocarbons (in 2006 – 3.8 %, in 2007 – 4.2 %) as well as oxygenated compounds (in 2006 – 16.1 %, in 2007 – 17.5 %) and sesquiterpene hydrocarbons (in 2006 – 6.7 %, in 2007 – 19.2 %) as well as their oxygenated compounds (in 2006 – 12.7 %, in 2007 – 13.4 %) in the essential oils of healthy linden blossoms increased.

Significant difference in the contents of oxygenated compounds (monoterpenes, sesquiterpenes, aliphatics, etc.) was determined in all the investigated samples of essential oils. The blossom oils collected from healthy linden contained from 47.3 % to 55.0 % of oxygenated hydrocarbons, and it was lower as compared to the amount found in the oils from the affected small-leaved linden (from 30.0 % to 15.3 %).

For the comparison, a relative alteration of the compounds of essential oils of small-leaved linden blossoms in healthy and damaged by fungal pathogens linden trees in 2006-2007 was calculated (Fig. 15).

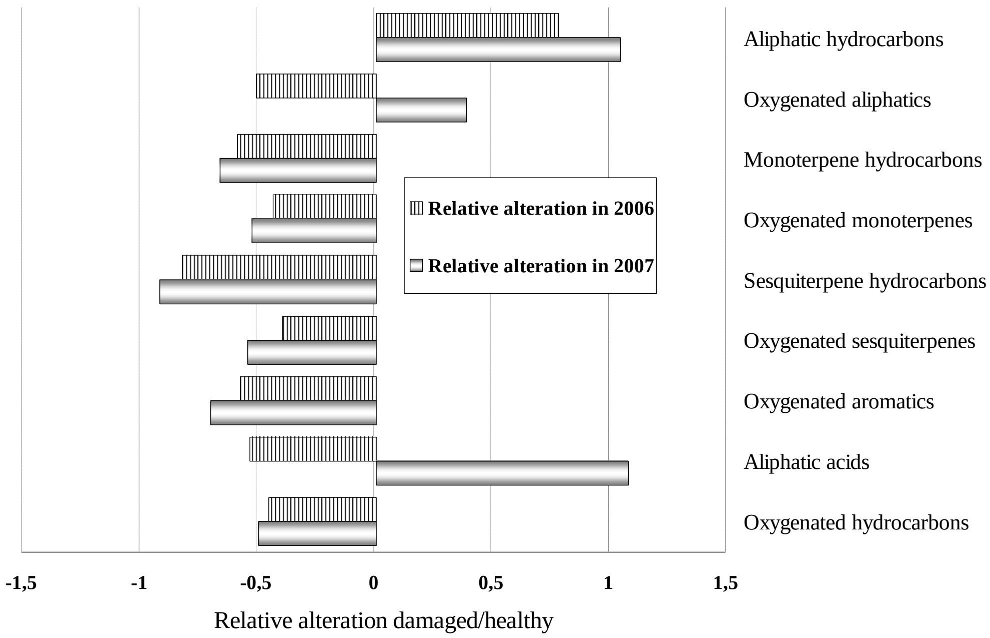


Fig. 15. Relative alteration of the compounds of essential oils of healthy and damaged by fungal disease agents small-leaved linden blossoms in 2006–2007.

In 2007, the difference in the data on relative alteration of aliphatic acids was revealed.

Supposedly, the synthesis of aliphatic acids might be also impacted by other factors – growth and meteorological conditions and other reasons.

Chemical composition of essential oils of small-leaved linden blossoms depends upon the impact of fungal disease agents. According to the chemical composition of essential oils of the blossoms we might assess the phytosanitary state of linden trees.

IMPACT OF BACTERIAL ISOLATES T_X AND U_X AND SACCHAROMYCES CEREVISIAE KILLER STRAINS UPON THE DEVELOPMENT OF PATHOGENIC FUNGI *IN VITRO*

Fungal disease agents on plants are widely distributed and the variety of caused diseases is large, therefore, a search for new plant protection measures is an important issue.

Killer activity of T_x and U_x strains (isolated from fruit and berries spontaneous ferment as well as standard *Saccharomyces cerevisiae* strains K7, M437, Rom-K100 on sensitive *Saccharomyces cerevisiae* α‘1 strain was examined. The strains T_x and U_x as well as control *Saccharomyces cerevisiae* standard K7, M437, Rom-K100 killer strains formed lysis zones. Basing on these studies, the impact of toxins of bacterial strains T_x and U_x as well as *Saccharomyces cerevisiae* killer strains upon pathogenic fungi of *Alternaria*, *Cytospora*, *Fusarium*, *Phomopsis* genera was tested.

It was established that after immediate transferring of T_x and U_x on MB (pH 4.8) medium surface lawn right away, T_x grew intensively and formed ~ 15 mm lysis zone. U_x growth was less intensive, however, it forms a larger lysis zone than T_x (Table 4).

Table 4. Impact of bacterial isolates T_x and U_x as well as *Saccharomyces cerevisiae* killer strains upon fungal disease agents of *Alternaria, Fusarium, Cytospora* and *Phomopsis* genera on MB (pH 4.8) medium

Taxa	Bacterial isolates		*Saccharomyces cerevisiae* killer strain		
	Tx	Ux	K7	Rom-K100	M437
Alternaria alternata	++	+++	-	-	-
Fusarium solani	++	+++	-	-	-
Fusarium sporotrichioides	++	+++	-	-	-
Cytospora leucosperma	+	++	-	-	-
Phomopsis velata	+	++	-	-	-

The size of lysis zone (mm) after immediate transferring of the culture: +++(>15mm); ++(>10 mm); + (<10 mm); ,,-" only contact inhibition

Two days after T_x and U_x transferring distinct lysis zones did not form. They were similar to control yeast strains, which showed only contact inhibition like after immediate transferring. In the case of deep lawn, T_x lysis zone remained similar, whereas that of U_x – twice longer because of a higher activity of the toxin secreted by U_x.

The nutritious media MB, YEPD and MEA were used for the research. To test killer activity of the toxins, the lawns of *S. cerevisiae* strain α'1 were employed for the control.

Deep lawns of *Alternaria alternata* as well as surface and deep lawns of *Fusarium solani* and *F. sporotrichioides* on MB medium were used. Yeast killer strains as well as bacterial isolates T_x and U_x were transferred immediately and after the appearance of fungal mycelium (after two days).

It was demonstrated that yeast killer strains K7, Rom-K100, M437, MS300 were not perspective for plant protection against the above-mentioned pathogens. Bacterial isolate U_x was proved to be the most perspective, however, a more detailed investigation on its growth conditions is necessary. Preliminary studies show that it is easier to apply the bacterial isolate T_x, because it grows and produces toxin on all three media – MB, YEPD and MEA. Due to specific features of fungi growth, in comparison with yeast, only approximate size of lysis zones might be determined (Table 4).

Strong impact of toxin-producing bacterial isolate U_x upon *Cytospora leucosperma, Phomopsis velata, Fusarium solani* and *F. sporotrichioides* fungal disease agents was revealed. Killer activity of bacterial isolate T_x in all cases was less intensive.

The toxins produced by the bacterial isolates T_x and U_x destroyed small-leaved linden pathogens *Cytospora leucosperma, Fusarium solani, F. sporotrichioides* and *Phomopsis velata* on all the investigated media (YEPD, MEA, MB), however, variation in their activity due to media composition and pH impact was demonstrated. Toxin-producing bacterial isolates T_x and U_x showed the highest killing activity during intensive growth of fungal mycelium. U_x produced toxin more intensively, and its affect upon pathogens was stronger than that of T_x.

CONCLUSIONS

1. 70 species of pathogenic fungi were recorded on small-leaved linden (*Tilia cordata* Mill.) in Vilnius city in 2005–2007. Of these, to *Ascomycota* phylum belong 5 species, *Basidiomycota* – 24, *Zygomycota* – 2, anamorphic fungi group – 39 species and 7 taxa were identified to genus rank.

2. 21 species of fungi were recorded for the first time on small-leaved linden, among which 3 species belong to *Ascomycetes* class, 2 – *Basidiomycetes*, 13 – *Hyphomycetes*, 3 – *Coelomycetes*. New in Lithuania fungi species *Phomopsis irregularis* (Died.) Petr. and *Pseudomassaria chondrospora* (Ces.) Jacz. were ascertained.

3. The detected pathogenic fungi belong to 3 trophic groups: obligatory biotrophs – 7 species, facultative biotrophs – 30 and facultative saprotrophs – 33 species. Obligatory biotrophs were the most wide spread: *Passalora microsora* (frequency of records (R_f) 35 %), *Discula umbrinella* (R_f 19 %), facultative saprotrophs or biotrophs: *Fumago vagans* (R_f 40 %), *Schizophyllum commune* (R_f 7 %), *Thyrostroma compactum* (R_f 6 %), *Cytospora leucosperma* ($R_{f\,d}$ 3 %), *Phomopsis velata* (R_f 0,5 %).

4. Small-leaved linden disease intensity varies in different habitats. The damage of green plantings on streets is the most harmful (1–4 grades). In squares, parks, public gardens 1-2 grade damage disease intensity was ascertained. The trees were damaged mostly by *Cytospora leucosperma*, *Discula umbrinella*, *Fumago vagans*, *Passalora microsora*.

5. Development of fungal disease agents is more intensive, when average air temperature is about +15–30 °C and mean precipitation amount – 160–200 mm. The largest number of fungi species was determined in August–September of 2005–2007.

6. *Cytospora leucosperma* and *Phomopsis velata* fungi are pathogenic to small-leaved linden. After inoculation of healthy trees cultivated under artificial light in growth chamber and field conditions the necrotic wounds were increasing and in both cases were developing similarly. The bark necrosis symptoms were first observed after 14 days.

7. Optimal growth and sporulation of the mycelium of pathogenic fungi *Cytospora leucosperma* and *Phomopsis velata* was demonstrated *in vitro* on malt extract agar (MEA) and malt extract peptone agar (MEPA) media. The impact of changes in hydrogen ions concentration, temperature and light is not significant; the fungi develop under wide range of media pH (5–9) and temperature (from +10 °C to +30 °C), by light and in the dark.

8. Pathogenic fungi *Passalora microsora*, *Discula umbrinella* and *Thyrostroma compactum* (=*Stigmina compacta*) affect biochemical processes in small-leaved linden. The chemical composition of essential oils of small-leaved linden blossoms changes – the content of aliphatic hydrocarbons increases and the amount of mono- and sesquiterpene hydrocarbons as well as their oxygenated compounds significantly decreases.

9. Toxins secreted by bacterial isolates T_x and U_x kill the mycelium of the pathogenic fungi species belonging to *Alternaria*, *Cytospora*, *Fusarium* and *Phomopsis* genera growing *in vitro*. The affect of toxin secreted by U_x is stronger than that of T_x.

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SANTRAUKA

Mieste želdiniai atlieka apsaugines, estetines, rekreacines bei kitas funkcijas, turi didelę įtaką žmonių gyvenimui, klimatui. Vilniaus miesto želdiniuose auginama per 100 rūšių sumedėjusių augalų, keturi iš jų liepos (*Tilia* L.) genties: mažalapė – *T. cordata* Mill., didžialapė – *T. platyphyllos* Scop., rečiau europinė – *T. europaea* L., grakščioji – *T. euchlora* K. Koch, kurios sudaro apie 80 % visų želdinių (NAVYS, 2005). Parkuose, skveruose, bendruomeniniuose kiemuose, gatvių želdiniuose, žaliųjų vejų juostose dažniausiai auginama mažalapė liepa, savaime paplitusi visame Baltijos regione (NAVASAITIS ir kt., 2003).

Miestuose įvairūs abiotiniai veiksniai (oro tarša, žiemą barstomos druskos, mechaniniai sužalojimai, nepakankama šaknų aeracija ir kt.) blogina medžių būklę (HARIS et al., 1999; GRIGALIŪNAITĖ ir kt., 2005b). Nusilpę jie tampa neatsparūs grybinėms ligoms (MONTAGUE et al., 2000; DONALD et al., 2001; SÆBØ et al., 2003; BÜHLER, 2006). Patogeninių grybų sukeltos ligos sumažina medžių dekoratyviąją ir rekreacinę vertę, sutrumpina jų amžių (LONSDALE, 1999; MORTIMER, KANE, 2004).

Želdinių patogeninių grybų tyrimai padeda įvertinti augalų fitosanitarinę būklę ir tinkamai juos parinkti miestų apželdinimui (TIKHOMIROVA, TOBIAS, 1999; KOLEMASOVA, KOVALEVSKAJA, 2000). Miestų želdiniuose nustatomos dažniausios grybinės ligos: lapų dėmėtligės, medienos puviniai, *Cytospora*, *Nectria*, *Phomopsis* genčių grybų sukeliamas šakų vėžys (BUTIN, KEHR, 1999; ADAMSKA, BŁASZKOWSKI, 2000; ADAMSKA, 2001; JUHÁSOVÁ, 2002; JUHÁSOVÁ et al., 2003). *Cytospora* genties grybai priskiriami prie miestuose dominuojančių grybų rūšių (TOBIAS, TIKHOMIROVA, 2006). Jie pažeidžia per 70 rūšių sumedėjusių augalų (KEPLEY, JACOBI, 2000; AGRIOS, 2005). Dėl želdinių grybinių ligų sukėlėjų daromos žalos iškyla nemažai problemų. Lietuvos mokslininkai iki 2005 m. ant liepų (*Tilia* L.) nustatė 185 rūšis grybų.

Mažalapė liepa yra jautri aplinkos pokyčiams, todėl tinkama miestų teritorijų gamtinei aplinkai vertinti (SNIEŠKIENĖ ir kt., 1999; ŽEIMAVIČIUS ir kt., 2003). Norint nustatyti neigiamų veiksnių poveikį liepoms, tikslinga būtų ištirti jų žiedų eterinių aliejų cheminę sudėtį, nustatant eterinius aliejus sudarančių komponentų skirtumus sveikuose ir pažeistuose grybais augaluose.

Urbanizuotoje teritorijoje cheminės apsaugos priemonės prieš ligų sukėlėjus nenaudotinos, taikintinos tik sanitarinės higieninės ir biologinės priemonės. Todėl prieš patogeninius grybus ir bakterijas ieškoma naujų, pasižyminčių antimikrobiniu aktyvumu, biologinių medžiagų. Tuo tikslu tiriami mielių bei kitų mikroorganizmų sekretuojami toksinai, kurie galėtų būti panaudoti patogenų sukeltai infekcijai augaluose lokalizuoti (MAGLIANI et al., 1997; WEILER et al., 2002; DONINI et al., 2005; MELVYDAS ir kt., 2006a).

Vilniaus mieste vyrauja mažalapė liepa, todėl tyrimo objektu pasirinkti patogeniniai grybai.

Liepų grybinių ligų sukėlėjų tyrimai urbanizuotoje teritorijoje yra aktualūs tiek teoriškai, tiek praktiškai – diagnozuojant liepų grybinių ligų sukėlėjus, įvertinant jų poveikį augalams bei siekiant taikomųjų tikslų – ieškant biologinių apsaugos priemonių prieš liepų patogenus.

Darbo tikslas – ištirti mažalapės liepos (*Tilia cordata* Mill.) patogeninių grybų paplitimą Vilniaus mieste, jų biologines savybes ir sukeliamas ligas.

Darbo uždaviniai:

1. Nustatyti mažalapės liepos patogeninių grybų rūšių įvairovę, paplitimą įvairiose Vilniaus miesto vietose bei jų vystymosi ypatybes.
2. Įvertinti mažalapės liepos grybinių ligų intensyvumą.
3. Ištirti žalingiausių mažalapei liepai grybų augimo *in vitro* ypatumus bei nustatyti jų patogeniškumą dirbtinio apšvietimo auginimo kameroje ir lauko sąlygomis.
4. Nustatyti kai kurių patogeninių grybų poveikį mažalapės liepos žiedų eterinių aliejų cheminei sudėčiai.

5. Ištirti antagonistinių bakterijų ir *Saccharomyces cerevisiae* kilerinių kamienų poveikį mažalapės liepos grybinių ligų sukėlėjams ir įvertinti jų praktinio panaudojimo galimybes.

Darbo naujumas. Nustatyta mažalapės liepos (*Tilia cordata*) patogeninių grybų taksonominė įvairovė Vilniaus mieste. Pirmą kartą Lietuvoje ant mažalapės liepos aptikta 21 rūšis grybų. Dvi rūšys *Phomopsis irregularis* ir *Pseudomassaria chondrospora* yra naujos rūšys Lietuvoje. Sudarytas sistematinis aptiktų grybų taksonų konspektas. Nustatytas mažalapės liepos patogeninių grybų rūšių paplitimo dažnumas. Įvertintas mažalapės liepos grybinių ligų intensyvumas. Ištirti mažalapei liepai žalingiausių patogeninių grybų augimo *in vitro* ypatumai ir nustatytas jų patogeniškumas dirbtinio apšvietimo kameroje ir lauko sąlygomis. Pirmą kartą atlikta liepų, pažeistų patogeninių grybų *Discula umbrinella, Passalora microsora, Stigmina compacta,* žiedų eterinio aliejaus komponentų pasiskirstymo chromatografinė analizė. Įvertintas šių grybų poveikis mažalapės liepos žiedų eterinio aliejaus cheminei sudėčiai. Nustatytas bakterijų kilerinių kamienų Tx ir Ux poveikis *Alternaria, Cytospora, Fusarium* ir *Phomopsis* gentims priklausančioms rūšims.

Darbo reikšmė. Sudarytas mažalapės liepos Vilniaus mieste ištirtų grybų taksonų sistematinis konspektas, papildantis kitų tyrėjų esamus duomenis. Patogeninių grybų *Cytospora leucosperma* ir *Phomopsis velata* biologinių savybių tyrimai papildo žinias apie šiuos grybus Lietuvoje. Sukaupti mažalapės liepos grybų tyrimo duomenys gali būti panaudoti rengiant daugiatomį leidinį „Lietuvos grybai“.

Įrodėme, kad patogeniniai grybai *Passalora microsora, Discula umbrinella* ir *Stigmina compacta* daro įtaką liepose vykstantiems biocheminiams procesams – keičiasi žiedų eterinių aliejų cheminė sudėtis. Pagal eterinio aliejaus cheminę sudėtį būtų galima įvertinti augalų fitosanitarinę būklę.

Bakterijų kilerinių kamienų Tx ir Ux lizuojančio poveikio mažalapės liepos patogeniniams grybams: *Cytospora leucosperma, Fusarium solani, F. sporotrichioides, Phomopsis velata* įrodymas yra svarbus kuriant ir pritaikant naujas biologines apsaugos priemones. Mielių bei kitų mikroorganizmų išskiriamus toksinus būtų galima panaudoti patogenų sukeltiems infekcijos židiniams augaluose lokalizuoti.

Tyrimų duomenys pateikti Vilniaus miesto savivaldybės administracijos Miesto plėtros departamentui skirtai ataskaitai „Vilniaus miesto želdynų stebėsena ir atnaujinimas“ ir Aplinkos ministerijos leidiniui „Želdynų apsauga ir tvarkymas urbanizuotose teritorijose“ (su bendraautoriais).

Ginami teiginiai. 1. Urbanizuotoje aplinkoje mažalapę liepą (*Tilia cordata*) parazituoja skirtingų sistematinių grupių grybai, iš jų daugiausia anamorfiniai grybai. 2. Liepų citosporozės ir smulkiaspuogio sukėlėjai *Cytospora leucosperma* ir *Phomopsis velata in vitro* gerai vystosi šviesoje ir tamsoje esant plačiam terpių pH ir temperatūrų diapazonui, yra patogeniški mažalapei liepai dirbtinio apšvietimo auginimo kameroje ir lauko sąlygomis. 3. Patogeniniai grybai *Discula umbrinella, Passalora microsora* ir *Stigmina compacta* turi poveikį mažalapės liepos žiedų eterinių aliejų cheminei sudėčiai. 4. *Alternaria, Cytospora, Fusarium* ir *Phomopsis* gentims priklausančių rūšių micelį, augantį *in vitro*, lizuoja bakterijų kileriniai kamienai Tx ir Ux.

Tyrimų medžiaga ir metodai. Mažalapės liepos patogeniniai grybai rinkti 2005-2007 m. Vilniaus mieste. Iš viso stebėta 5113 medžių. Grybų grynų kultūrų išskyrimui šakelių ir lapų pavyzdžiai sterilinami pagal T. N. SIEBER ir kt. (1991) metodiką. Makromicetų pavyzdžiai identifikuoti pagal vaisiakūnių morfologines savybes. Grybų rūšys nustatytos remiantis įvairių autorių darbais. Grybų rūšių identifikavimui polimerazės grandininės reakcijos (PGR) metodu, DNR išskirta naudojant rinkinį NucleoSplin®Plant II (gamintojas GmbH&Co MACHEREY-NAGEL, Vokietija). Gautos tiriamo grybo DNR sekos palygintos su sekomis, esančiomis elektroninėje Nacionalinės biotechnologinės informacijos centro (NCBI) Genų banko duomenų bazėje (ALTSCHUL et al., 1997). Meteorologinės sąlygos įvertintos pagal Meteorologinio biuletenio duomenis (SKEIVELIENĖ, 2005, 2006, 2007). Mikromicetų vystymasis tirtas ant skirtingų agarizuotų mitybinių terpių: salyklo ekstrakto (SEA), salyklo ekstrakto peptono (SEPA), bulvių dekstrozės (PDA), kukurūzų (CMA), Čapeko-Dokso (ČDA), Hagemo (HA). Vandenilio jonų koncentracijos įtaka mikromicetų augimui tirta naudojant SEA terpę, kurios pH 3, 4, 5, 6, 7, 8, 9, 10. Temperatūros ir šviesos režimo įtaka kolonijų augimo greičiui, tirta kultivuojant mikromicetus ant SEA terpės +5 ºC, +10 ºC, +15 ºC, +20 ºC, +30°C temperatūroje, šviesoje ir tamsoje. Grybų patogeniškumas tirtas dirbtinai užkrėtus jais mažalapes liepas, kultivuotas auginimo kameroje ir lauko sąlygomis. Dirbtinis užkrėtimas atliktas pagal D. P. WEINGARTNER ir E. J. KLOS (1975) metodiką. Mažalapės liepos žiedų eteriniai aliejai išskirti hidrodistiliacijos būdu. Jų cheminė sudėtis nustatyta dujų chromatografijos-masių spektrometrijos metodais. Eterinio aliejaus junginiai identifikuoti pagal junginių sulaikymo kolonėlėje koeficientus (IR) bei masių spektrus, lyginant juos su etaloninių junginių sulaikymo duomenimis bei masių spektrais (DAVIES, 1990; ADAMS, 2001). Mielių kontrolinių kilerinių kamienų *Saccharomyces cerevisiae* K7, Rom-K100, M437 ir bakterijų izoliatų Tx ir Ux (išskirtų BI Genetikos laboratorijoje) kilerinis aktyvumas nustatomas pagal testuojamų kamienų gebėjimą suformuoti lizės zonas ant testerinio *Saccharomyces cerevisiae* α’1 kamieno giluminio gazono (SHERMAN, 1986; GULBINIENĖ ir kt., 2004; MELVYDAS ir kt., 2007). Skirtingų substratų grybų sudėčių panašumui įvertinti naudotas Sørensen’o indeksas (Sørensen index, C_S) (SØRENSEN, 1948). Grybų rūšių aptikimo dažnis (A_d) apskaičiuotas pagal CASTILLO ir kt. (2004). Vidutinis pažeidimo balas grybinių ligų intensyvumui įvertinti apskaičiuotas pagal ŠURKUS ir GAURILČIKIENĖ (2002).

Duomenų statistinė analizė atlikta pagal V. ČEKANAVIČIAUS ir G. MURAUSKO (2002) metodines rekomendacijas, pasinaudojant SPSS 16.0 versiją ir Microsoft® Office Excel 2003 (Statistical Analysis ToolPak) programomis.

Tyrimų rezultatai. 2005-2007 m. Vilniaus miesto želdiniuose ant mažalapės liepos (*Tilia cordata* Mill.) nustatyta 70 patogeninių grybų rūšių, priklausančių *Ascomycota* skyriui – 5 rūšys, *Basidiomycota* – 24, *Zygomycota* skyriui – 2 bei anamorfinių grybų grupei – 39 rūšys ir 7 taksonai apibūdinti iki genties rango.

Pirmą kartą Lietuvoje ant mažalapės liepos nustatyta 21 rūšis grybų, iš kurių 3 priklauso *Ascomycetes* klasei, 2 – *Basidiomycetes*, 13 – *Hyphomycetes*, 3 – *Coelomycetes* klasei. Išaiškintos naujos Lietuvai grybų rūšys *Phomopsis irregularis* (Died.) Petr. ir *Pseudomassaria chondrospora* (Ces.) Jacz.

Nustatyti patogeniniai grybai priklauso trims trofinėms grybų grupėms: obligatiniai biotrofai – 7 rūšys, fakultatyviniai biotrofai – 30 ir fakultatyviniai saprotrofai – 33 rūšys. Labiausiai išplitę obligatiniai biotrofai: *Passalora microsora* (aptikimo dažnis (A_d) 35 %), *Discula umbrinella* (A_d 19 %); fakultatyviniai saprotrofai ar biotrofai: *Fumago vagans* (A_d 40 %) *Schizophyllum commune* (A_d 7 %), *Thyrostroma compactum* (A_d 6 %), *Cytospora leucosperma* (A_d 3 %), *Phomopsis velata* (A_d 0,5 %).

Mažalapių liepų ligų intensyvumas skiriasi įvairiose augavietėse. Labiausiai pažeisti gatvių želdiniai, kuriuose nustatytas 1-4 balų ligų intensyvumas. Aikštėse, parkuose, skveruose ligų intensyvumas 1-2 balai. Daugiausia medžius pažeidė *Cytospora leucosperma*, *Discula umbrinella*, *Fumago vagans*, *Passalora microsora*, *Thyrostroma compactum*.

Grybinių ligų sukėlėjų vystymasis intensyvesnis, kai vidutinė oro temperatūra yra apie +15-30ºC ir vidutinis kritulių kiekis 160-200 mm. Daugiausia grybų rūšių nustatyta 2005-2007 m. rugpjūčio-rugsėjo mėnesiais.

Citosporozės bei smulkiaspuogio sukėlėjai *Cytospora leucosperma* ir *Phomopsis velata* yra patogeniški mažalapei liepai. Inokuliavus šiais patogenais sveikas liepas, augančias dirbtinio apšvietimo auginimo kameroje ir lauko sąlygomis, nekrozės didėjo ir abiem atvejais plito panašiai.

Patogeninių grybų *Cytospora leucosperma* ir *Phomopsis velata* micelio augimui ir sporuliacijai *in vitro* optimaliausios yra salyklo ekstrakto agaro (SEA) ir salyklo ekstrakto peptono agaro (SEPA) mitybinės terpės. Šie grybai mažai jautrūs terpių vandenilio jonų koncentracijos, temperatūros ir apšvietimo pokyčiams, gerai vystosi plačiuose terpių pH (pH 5-9) ir temperatūrų (+10-+30ºC) diapozonuose, šviesoje ir tamsoje.

Patogeniniai grybai *Passalora microsora*, *Discula umbrinella* ir *Stigmina compacta* turi įtakos liepose vykstantiems biocheminiams procesams. Pakinta žiedų eterinių aliejų cheminė sudėtis – juose daugėja alifatinių angliavandenilių ir ženkliai mažėja mono- ir seskviterpeninių angliavandenilių bei jų oksiduotų junginių.

Bakterijų kamienų Tx ir Ux sekretuojami toksinai lizuoja *Alternaria*, *Cytospora*, *Fusarium* ir *Phomopsis* gentims priklausančių rūšių micelį, auganti *in vitro*. Kamieno Ux sekretuojamo toksino poveikis patogeniniams grybams yra stipresnis nei Tx.