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Oaks Decline in the North and West of Transylvania

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Abstract

The basic concept on which are ruled the forests in Romania is the one of realizing a stable forestry. An important way to achieve this objective is to stop the quercines wither. This can be done by carefully studying of the biotic and non-biotic factors. The present work proposes a clear-up concerning the involvement of the biological factors, i.e. the micromycetes, in the oak decline. This can be done by carefully studying of the biotic and non-biotic factors. Despite the researches that have been developed, of numerous studies and analysis done by different experts in forestry, biology, chemistry, geology, environment protection etc., the problem is still far to be completely resolved, because of the complex characteristics of the causal agents. The forms of *Chalara* type are frequent found in flowers, acorns and wood from oak and sessile oak, they are a reality that we believe it must be considered, even if the species of which they belong are not always rigorous identified. It remains to establish in the future their pathogenic power, also much controvert. We also consider that it is necessary to give an increased attention to the competitive saprophyte fungi, to eventually use them in biological pest control.

Keywords: oak, stands, decline, fungus, *Chalara, Ceratocystis*

1. Introduction

The basic concept on which are ruled the forests in Romania is the one of realizing a stable forestry. An important way to achieve this objective is to stop the quercinees wither. This can be done by carefully studying of the biotic and non-biotic factors. The present work proposes a clear-up concerning the involvement of the biological factors, i.e. the micromycetes, in the oak decline [12, 15]. The forestry stands area in the North-Western part of Romania is about 1,370 thousand hectares, from which the quercinees stock is about 356 thousand hectares (26%). The oak decline occurs in all Forestry Departments, on variable areas and with different intensity degrees [9, 10, 11, 12].

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The most affected areas are in the forests belonging to the counties of Satu Mare (15388 Ha), Maramures (3939 Ha) and Bihor (2070 Ha).

Despite the researches that have been developed, of numerous studies and analysis done by different experts in forestry, biology, chemistry, geology, environment protection, etc., the problem is still far to be completely resolved, because of the complex characteristics of the causal agents [6, 7, 8]. Concerning the imply of the fungal diseases, the one of the most important biotic factors in the South - Eastern Europe (former Yugoslavia, Romania and former S.U.), was revealed, next to the slow decline of the oak, the phenomenon of fast wither, caused by some vascular species, belonging to *Ophiostoma (Ceratocystis)* genus, which identity and pathogenic activity remains uncertain and disputed, moreover about the possible occurring of the devastating species of *Ceratocystis fagacearum*, considered to be endemic in the U.S.A. The researches made in our country by C.Georgescu et al. [7, 8] lead to

identify some species of *Ophiostoma (Ceratocystis)* such as: *Ophiostoma roboris, Ophiostoma valachica*, also *Ceratostomella querci* and other fungus species that could be implied in the oak decline. A particular role belongs to the mentioned species, because of their systemic, vascular action [13, 20]. In certain conditions, the sudden wither of the oak is associated with the presence of the form *Chalara* type, very close to *Chalara quercina*, the anamorphed of the *Ceratocystis fagacearum*, who is responsible of the massive decline of the oak in U.S.A. [1, 2, 3, 4, 17]. As associated species are mentioned *Armillaria mellea*, which gives the wither process a more quick evolution, and *Phomopsis quercella*, with an undefined role.

The present researches, which are done in cooperation by experts from I.C.A.S. and U.S.A.M.V. Cluj-Napoca, bring new data about these aspects.

2. Material and Method

Along the researches, it was realized the isolation of oak and sessile oak wood fungus (by longitudinal and transversal sections) from trees under the withering process in the forests Baciu and Hoia (from Cluj Forestry Departament.). The sections, kept in sterilized, distilled water, was passed on agarized or liquid (beer juice, malt) cultural mediums, and for a better sporulation and eventually forming of peritecies was used the medium containing alanine, mentioned by True et al. [19]

From the same trees were isolated flowers and acorns in different stages of development, a part of this material becoming from the "Foieni" plantation, in Satu-Mare Forestry Departament., and seed reservation "Bavna" in Somcuta Mare Forestry Departament. Determinations were made on samples of female flowers of oak, from which were made isolations of brown tissues (ovary, ovules, pistil, stigma, peduncle) on Czapek-Dox medium.

3. Results amd Discussions

In 2007 were isolated some fungus colonies, in which was found anamorphes of *Graphium* and *Chalara (Endoconidiophora)* types, from 1 and 5 samples. In 2008, the isolates of *Chalara* types were numerous, realised on wood and flowers, from 10 samples. To these, were added 4 isolates with the conidian form of *Graphium*, 2 with *Chalaropsis* and one of each *Rhinotrichum* and *Hyalodendron* types, in the same time being made evident peritecies of *Ceratocystis* (samples 2, 4 and 13), directly on the wood or on the cultural medium (distilled water or beer juice and agar).

In 2009 were isolated forms of *Chalara* type from 4 samples of wood and acorns (samples 1, 25 and 27), a conidian form of *Hyalodendron* type (sample 28), 3 isolates of *Chalaropsis*, one of *Verticillium* (sample 27), and also peritecies of *Ceratocystis* type (samples 2 and 28).

These isolates are presented in table 1, and the morphological, biometrics and cultural characteristics are presented in tables 2 and 3.

Also were made evident mycelia conidies, hyphal buds, conidies of *Cephalosporium* and *Thielaviopsis* types.

All isolates grow and develop well in beer juice medium (liquid and agarized), on which conidies were formed abundantly, more numerous and more quickly eliminated from hyphes and conidiophores on the medium containing alanine.

The 6 isolates with anamorphes presented in tables 2 and 3 have all one common characteristic - the fact of forming endoconidies, at the 3, 4 and 5 isolates being the only conidian form which was present, since at the 1, 2 and 6 isolates was found other forms too.

The anamorphes of the last forms seem belonging to the species *C. major* (Hunt), *C. variospora*, *C. fagacearum ?I* (samples 6 and 4).

The anamorphes of *Graphium* and *Hyalodondron* types were obtained in culture, together with peritecies of *Ophiostoma (Ceratocystis) roboris*, their link being evidently proved. The telemorphes (peritecies) observed directly on the wood were obtained on wood cultures in water, only one on beer juice and agar (sample 4) and belong to the species: *Ceratocystis piceae, C.cariospora, C. cappillifera, C. (Ophiostoma) roboris*.

Obtaining the conidian forms of *Rhinotrichum* and *Verticillium* type in culture, we can suspect the presence of the species *Ophiostoma (Ceratocystis) valachicum*, and *O. (Cerat.) kubanicum*, the last one being signaled in formal USA too.

Together with the mentioned isolates which were obtained - in parallel - many accompanying species, competitive, some with a very accentuated antagonistic character, such as: *Trichoderma viride* (most frequent), *Coniophora sp., Innonotus sp., Pholiota sp., Alternaria alternata, Epicoccum purpurascens* and *Fusarium sp.*

Moreover the first 5 species made very difficult the obtaining of pure cultures, because they grow much faster than the species of *Ceratocystis*, which are sometimes totally invaded.

Table 1. Anamorphes and telemorphes isolates belonging to species of *Ceratocytis* involved in the oak, during 2007-2009

S. no	2007		2008				2009			
	Chal (Endoc.)	*Graph.*	*Chal (Endoc.)*	*Graph.*	Other conid.	*Cerato-cystis*	*Chal*	*Graph.*	*Other forms*	*Cerato-cystis*
1	W X	W X	W X	W VI			W X			
2			F VI	W VI		W X *C.cap.*				W I *C.cap.*
3			F VI	W VI			F VI			
4				W IX	W IX *Hyal.* *Ceph.* *Rhin.*	W VI *C.p.* W X *C.r.*				
5	W X		W X		W X *Chp.*					
11			W X							
12			W X							
13			W IX		W IX *Chp.*	W X *C.v.*				
18			F VII						F VI *Chp.*	
19			F VII						F VI *Chp.*	
25			W IX				W X A X		F VI *Chp.*	
27							W XI		W X Vrt.	
28								W VI	W VII *Hyal.*	W I *C.r.*

Legend: I-X = January - October; W = wood; F = flower; G = acorn; *C.p. = Ceratocystis piceae; C.r. = C. roboris; C. cap. = C. capilifera; C.v. = C. variospora; Chal. = Chalara; Endoc. = Endoconidiophora; Hyal. = Hyalodendron; Graph. = Graphium; Chp. = Chalaropsis; Vrt. = Verticillium; Ceph. = Cephalosporium; Rhin. = Rhinotrichum.*

Table 2. Characters of some isolates belonging to some species of *Ceratocystis*

Isolate/Issue	1	2	3	4	5	6
Isolating substrate	Sessile oak wood (no.1 - 4) mycelium and white hyphes bouquets	Oak wood (no. 13, 25) Oak flower - ovary, browned bucket (no.18, 9)	Seessile oak flower (no. 3) The same, oak	Sessile oak wood	Sessile oak acorn	Sessile oak sapling
Culture medium	Malt-agar and alanine medium	Malt-agar and alanine medium	Malt-agar and alanine medium	Beer juice - agar	Beer juice - agar	Malt-agar
The colony aspect	Fluffy-wooly, concentric zoning	Felt-thick,	Fluffy, compact on the walls, honeycomb-like in the middle	Felt-thick, raising on the walls	Felt-thick, waved	Wooly, with conidies gatherings
The colony color	Creamy-white to grey-yellow	Creamy-white to brown	Orange-yellow with brown-green shadows	Dark grey	White-grey, black in the middle	Grey-white
The back of colony color	Brown-black	Creamy with brown rays	Brown - dark red	Black	Black	Grey
Growing speed	3.4 mm / 24 hrs.	10 mm / 24 hrs.	7.4 mm / 24 hrs.	5.5 mm / 24 hrs.	1.2 mm / 24 hrs.	2.5 mm / 24 hrs.
Hyphes shape and diameter	Straight angle ramifications, anastomosis, curls, cords (2 - 3 hyphes); 2.5 – 7.5 µm diam.	Straight angle ramifications, curves, curls, some open at the end; 2.5 - 6 µm diam.	Straight angle ramifications, waved hyphes, cords; 1.,2 - 5 µm diam.	Straight angle ramifications, contortions	Straight angle ramifications, curls	Straight angle ramifications,, anastomosis
Hyphes color	Hyalin-yellow, brown	Hyalin-yellow, brown	Hyalin-yellow, brown	Hyalin	Hyalin	Hyalin-yellow

Table 2 - continued

Isolate/Issue	1	2	3	4	5	6
Conidiophores	Typical for *Graphium*. hyphes with endoconidies	Non-differentiate. ribbon shape. 50-125 µm long	Non-differentiate	Small differentiation. ribbon-like ends. 87.5 - 225 µm long	Bottle-like shape. brown hyalin. 32.5 - 115 x 2.5 - 5 µm	Small differentiation. hyalin. yellow-brown
Types of conidies	- mycelian - *Graphium* - endoconidies of 2 types	2 types endoconidies. rectangulares. ovoid. *Chalaropsis* type	Mycelian. endoconidies	Hyphal buds and endoconidies. rectangulares. hyalin pale-yellow	Hyphal buds and endoconidies. rectangulares. hyalin	2 types endoconidies: - ovoid hyalin and rectangular. yellow - ovoid pyriform *Chalaropsis* type
Shape and color of conidies	- pyriform. hyalin - ovoid elongated. straight and curved - rectangulare and ovoid. in chains. hyalin. yellow-brown	- rectangulare. mucilaginous layer around. in chains. hyalin. yellow; - ovoid. yellow-brown - spheric. brrown	- c.m. ovoid yellow - e. rectangulare. yellow-orange. in chains			
Size of conidies	c.m. 7.5-22.5 x 3.1-5 µm; Gr. 3.1-11.2 x 1.9-3.7 µm; e. 8.7-12.5 x 3.7-6.2 µm	3.1-7.5x 1.9-3.7µm 6.2-25 x 3.1-3.7µm 11.2-12.5 µm diam.	e. 16.2 x 3.7 µm e. 3.1-5.6 x 1.9-2.5 µm	e. 6.2-25 x 3.1-3.7 µm e. 7.5-17.5 x 3.1-3.7 µm (intercalated)	2.5-8.7 x 1.9-2.5 µm	5-5.6 x 2.5 µm 8.7-23.7 x 11.2 µm
Observations	No peritecies formed	No peritecies formed	No peritecies formed	No peritecies formed	No peritecies formed	No peritecies formed
Affiliation to species	*C. major* ?	*C. variospora* ?	?	*C. fagacearum* ?	?	?

Table 3. Telemorphes of some *Ceratocystis* species on sessile oak and oak

Isolate/Issue	1	2	3	4
Formation place	sessile oak bark (s.4)	Oak wood (s.13), sterilised water culture	Sessile oak wood (s.2)	Malt-agar medium inoculated with sessile oak wood (sterilised water culture)
General aspect	After a month from collecting, on the tracks left by insects in wood developed a rich mycelian tissue, with greenish rings, on which was formed nomerous peritecies, long-necked	On the wood piece but also in water and on the walls was formed globular peritecies with a long enough neck	In humidity conditions developed superficial peritecies on a white-creamy mycelium to yellow-brown	Mycelian colony grey-black with white conidies islands *Hyalodendron* type of 5-16.2 x 2.5-3.7 µm and *Graphium* type of 3.1-7.5 x 1.9-3.7 µm
Shape, color and diameter of peritecies basis	Spheric, brown-black; 150-295 µm	Globular, dark brown-black, covered with brown hair, non-sected; 82.5-344.4 µm	Spheric flattened at the base, brown-black; 125-184 µm	Hemisperic, relative long neck, brown-black 200-246 µm
Shape and dimensions of peritecies neck	Elongated, sharpened at the end; 553-664 x 12.3-13,7 µm (at the top) and 36.9-49.2 µm (at the base)	Elongated, straight, flexible, narrowed to the top, brown at the base, hyalin-yellow at the top; 37,3-553 µm, media of 261 µm x 12,5-15 µm (at the top), 24-37,5 µm (at the base)	Elongated, truncated at the end, dark brown, ostiolar hyphes, thin, hyalin. Neck of 246-1845 x 36,9-49,2 µm (at the base), 24,6 µm (at the top)	Elongated, brown, at the top almost hyalin; 590-910 x 10-12.5 µm (at the top), 25 µm (at the base)

Table 3 - continued

Isolate/Issue	1	2	3	4
Peritecies ornaments	Hyphes on the neck and at the top, few basal hyphes, 72.5 x 5 µm	Brown hyphes, more at the base, 20-175 x 1.9-2.5 µm, at the top hyalin hyphes	Very few hyphes at the base	At the top a crown of about 20 hyalin hyphes, sectional, 20-30 µm long (at the majority not observed)
Shape and size of the ascus	Ovoid, 32.5 x 12.5 µm	Not seen	Not seen	Not seen
Shape and size of the ascospores	Ovoid, reniform, hyalin, 3-3.2 x 1.9-2.5 µm	Ovoid, sometimes flattened on a side, hyalin 3.1-6.2 x 2.5-3.7 µm	Not seen	Allantoids, hyalin, 3.1 x 1.9-2.5 µm
Species	*Ceratocystis piceae* (Munch.) Bakshi	*Ceratocystis variospora* (Dav.) (syn. *C. fimbriata*) Moreau	*Ceratocystis capilifera* (Hedgc.) Moreau (syn. *Ceratostomella longirostellata* Bakshi)	*Ophiobolus (Ceratocystis) roboris* Georg. et. Teod. (*C. piceae* ?)

In association we also identified the species: *Valsa ceratophora, Acropeira mirabilis, Phialophora fastigiata* and very frequent *Penicillium sp.*

4. Conclusions

On the trees which were under the decline (wither) process, we could observe a complex of fungus species, through which was invariable identified species belonging to genus *Ceratocystis*, conidian forms and peritecies (telemorphes).

From the anamorphes, we consider the most important the ones of *Chalara (Endoconidiophiora)* type, different by morphological and cultural point of view, belonging to different species, uneasy to identify because of some characteristics which are not enough evident, in the determining catalogues (charts) available to us [8, 18], being accentuated the perfect forms, but the species characteristics are often interpenetrated.

On the other side, the competitive organisms made very difficult to obtain pure cultures. The success of the isolations is very much conditioned by the maintaining of the humidity in the mediums - isolates from dry wood being with no result.

Morphologic variations between isolates of *C. fagacearum* were reported by Henry [19], proving that some cultures produce white mycelium after they have been cultivated a long period (moreover the unisexual male mycelium).

Knowing and comparing the oak decline symptoms in the North-West Romanian forests with the one produced by *C. fagacearum* in U.S.A.; and comparing the morphologic characters of anamorphic isolates of *Chalara* type, where was nor made evident other conidious forms and neither peritecies, moreover the great variability of the species belonging to *Ceratocystis* genera, we can assume that between the species implied in the fast wither of the oak could be also some isolates obtained by us. For this supposition is pleading also some cultural and morphologic characters, considered by True et al. [19] as basic criterion in species determination, also considered by us, i.e.: ribbon like ending of the conidiophores, producing of numerous waved and curved branches on young mycelium, the size of the conidies, the color of the back of the colony etc.

Meanwhile we do not exclude the fact that other species, considered unimportant, can play an important role in pathogenesis, such as *C. roboris* or *C. piceae*, which can obtain virulence.

We consider that a more careful research is further necessary, to observe the microorganisms involved in the process of oak decline. Gibbs showed the complicated situation of the great similitude between the published descriptions for fungi, as: *C. querci, Ophiostoma (Cerat.) roboris* and *C. piceae*, revealing the necessity of a detailed study of the morphologic and cultural characters of each *Ceratocystis* species, without excluding the possibility to exist in Europe of *C. fagacearum*, showing that the white European oak can be less resistant than the American oak , and the climatic conditions in some parts of Europe can be more favorable. Moreover, it reveals the appearing risk of this species, by the presence in Europe of its vector, the insect *Scolytus intricatus* (Rottab.).

We believe that it must not be excluded the possibility of the spreading area extinction, and neither the existence for some new appeared races, in different climatic conditions.

The forms of *Chalara* type are frequent found in flowers, acorns and wood from oak and sessile oak, they are a reality that we believe it must be considered, even if the species of which they belong are not always rigorous identified. It remains to establish in the future their pathogenic power, also much controvert. We also consider that it is

necessary to give an increased attention to the competitive saprophyte fungi, to eventually use them in biological pest control.

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