MICROSTROMA ALBUM (DESM.) SACC. AND *MICROSTROMA JUGLANDIS* (BERENGER) SACC. IN NORTH WESTERN ROMANIA

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Abstract

Outbreaks of formerly endemic tree pathogens have increased in frequency and spatial extension during last decades due to climate change and anthropogenic stress. Microstroma album and Microstroma juglandis, obligate pathogens placed among Basidiomycota were studied in North-Western Romania between 2009 and 2014 when outbreaks were noticed in forest stands dominated by Quercus species (Q. cerris, Q. robur, Q. petraea, Q. pubescens) and in gardens, mainly in urban areas for Microstroma juglandis on Juglans regia. The associated foliar and shoot galling arthropods were considered and the trithrophic interaction (pathogens-hosts-galling insects) was incorporated in network formalism. Connectivity, betweeness centrality and normalized node degree were assessed as important network descriptor. Low connectivity (0.13), pathogens playing the role of strategic vertices mirrored by their high normalized node degree values, Q. petraea, Q. cerris, Q. robur and the obligate pathogen Microstroma album showing the highest values for betweeness centrality described the main characteristics of the network. Pathogen mediated structure of arthropod communities linked to particular tree species must be considered in planning sustainable control measures which must be based on sound ecological theory.

Key words: *Microstroma album, Microstroma juglandis*, trithrophic interactions, network metrics

INTRODUCTION

Outbreaks of formerly endemic tree diseases and the spread of emerging diseases of exotic tree pathogens represent one of the main consequences of globalized trade having as a natural background, climatic changes (Gonthier and Garbelotto, 2013; Santini et al., 2013; Stenlid et al., 2013). However, understanding the intricate system of interspecific interactions with hosts and other associated organisms is prerequisite condition for management and control of the diseases. During recent years, endemic pathogens showed the clear tendency of epidemic spread. It is expected that those species will interact with phytophagous insects and other pathogens in positive and negative ways (Hatcher, 1995; Stout et al., 2006), establishing complex networks. Pathogens have the potential to modify the structure of different plant feeding insects guilds: galling insects and mites, mining and free feeding insects or other pathogens (Stout et al., 2006; Tack et al., 2012).

Within the genus *Microstroma* (Basidiomycota:Exobasidiomycetes: Exobasidiomycetidae:Microstromales:Microstromataceae), 30 species of obligate pathogens were identified to date but the members of this taxonomic group are notoriously difficult to detect in the field (Begerow et al., 2001).

Outbreaks of *Microstroma album* (Desm.) Sacc. were historically reported in Poland, in 1924 (Zweibaumova, 1925) and more recently in New Zealand on *Quercus robur* and *Q. robur x Q. canariensis* (Braithwaite et al., 2007). It was reported on *Quercus robur, Quercus pubescens, Quercus macranthera* (in United States of America and in Botanical garden of Graz University, Austria) (Scheuer, 2004), on *Q. garryana* in Canada (DAVPF Collections database) on *Quercus lusitanica* in Algeria (Kew herbarium IMI, access number: 15992), as *Microstroma album* var *japonicum* Henn. on *Quercus acutissima* in Japan. The pathogen was mentioned by Kew Mycology, Species Fungorum in the Catalogue of Life as being present in Romania on *Q. frainetto* in Southern part of the country, in broadleaved forest stands in the area of the locality Vlad Țepeş, collected by Negrean G. in 1974 (specimen no 94642 in Kew Herbarium IMI).

Microstroma juglandis (Berenger) Sacc. is reported in Kew catalogue of Life as being identified in Germany, Great Britain, Greece, Hungary, Kashmir, India, Italy, Netherlands, USA and Romania, on *Juglans regia, Carya ovata, Juglans nigra, Carya tomentosa, Carya illinoensis, Hicoria glabr*a and is considered an invasive species (Anselmi, 2001) called yellow blotch or white mold. On *Carya ovata* causes the development of witches' brooms. It was reported in Europe, North America and Eastern Asia (Kurt et al., 2003; Garcia-Jimenez et al., 1995; Lee et al., 2011). Recently it was reported also from Slovenia (Orig and Jurc, 2013). In reported countries, the disease was considered of special concern for walnut growers. In Romania it was mentioned on *Juglans regia* by Săvulescu and Sandu-Ville in 1940 (Kew Herbarium IMI, no 15996), by Comes in 1964 in the locality Ocnele Mari (Kew Herbarium, IMI no. 135657) and more recently, in the metropolitan area of Oradea (Sturz and Cupșa, 2006). The species is included in the list of invasive species threatening natural and anthropogenic ecosystems of Europe (Drake, 2009).

The present paper reports the presence of the two *Microstroma* species in a relatively continuous area, in North-Western Romania, suggesting that the pathogens have reached an epidemic status during the last several years corresponding to the changing climate in the area and intensified anthropogenic stress. Associated with other foliar pathogens, insect pests and abiotic stressors, the species could contribute to the important shifts in community structure both of hosts and associated organisms and also decline in the area of the targeted host species. Ecological network formalism was employed to depict the relationships across organisms attacking the foliar system of *Quercus petraea, Q. robur, Q. cerris* and *Q. pubescens* in North-Western Romania. The analysis of ecological networks permits the visualization and exploration of interaction patterns (Jordano, 2010) addressing quantitatively the structure of these interactions assisted by specific network or graph metrics which describe locally (such as centrality metrics) or globally the networks (such as connectivity). The present study investigates the structure of tritrophic interactions (hosts-obligate foliar pathogens-galling insects) with a special emphasis on *Microstroma album*, a previously infrequent obligate pathogen on *Quercus* spp. Additional information on the presence of *Microstroma juglandis* in north-Western Romania is also included.

MATERIAL AND METHOD

Samplig areas

The diseased material consisting of leaves presenting the specific lesions and was collected during the summers of 2012, 2013 and 2014 in different locations of Cluj, Bihor, Alba and Sălaj Counties from Central and North-Western Romania, covering a transect of 170 km, 18 sampling sites near 10 urban and rural localities, in forest stands and private gardens (localizations indicated on the map, Fig. 1).

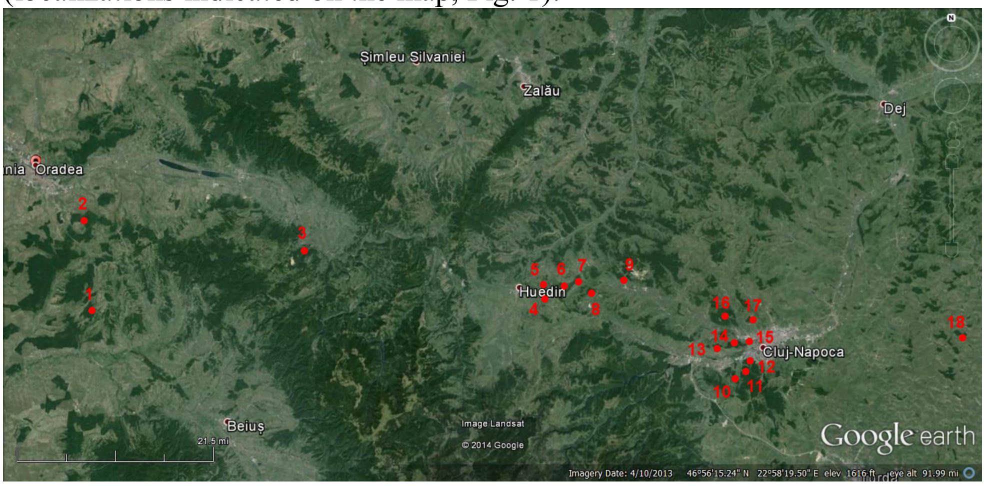

Fig. 1 Satellite map of the area in North-Western Romania containing the sampling locations (main cities: Cluj-Napoca, Huedin and Oradea) (Google Earth, 2014)

Locations:

1. Lăzăreni (46°50'40.10"N 22°02'53.49"E); **2.** Băile 1Mai (46°59'01.03"N 22°00'56.03"E); **3.** Şuncuiuş (46°56'11.77"N 22°31'57.60"E); **4.** Jebuc site 1 (46°51'36.09"N 23°07'56.16"E); **5.** Jebuc site 2 (46°52'02.07"N 23°06'48.52"E); **6.** Stana (46°52'29.92"N 23°08'57.06"E); **7.** Gălăşeni site 1 (46°53'00.42"N 23°10'06.19"E); **8.** Gălăşeni site 2 (46°51'54.10"N 23°11'22.45"E); **9.** Aghireşu (46°52'49.00"N 23°16'51.56"E); 10. Făget site 1 (46°43'31.33"N 23°30'57.97"E); **11.** Făget site 2 (46°43'56.75"N 23°32'14.99"E); **12.** Făget site 3 (46°44'48.24"N 23°32'49.55"E); **13.** Hoia site 1 (46°46'07.97"N 23°28'43.94"E); **14.** Hoia site 2 (46°46'10.18"N 23°30'59.34"E); **15.** Hoia site 3 (46°46'37.10"N 23°33'05.18"E); **16.** Baciu (46°47'16.49"N 23°29'38.42"E); **17.** Dâmbu Rotund (46°47'19.92"N 23°31'01.39"E); **18.** Mociu (46°46'34.35"N 24°02'08.08"E).

For *Microstroma album* leaves presenting characteristic lesions were collected from seedlings and mature trees of *Quercus robur, Q. cerris, Q. pubescens* and *Q. petraea.*

Microstroma juglandis on *Juglans regia* was observed in private gardens, in rural areas such as Baciu commune and in urban area Cluj-Napoca (2013), in Hoia recreational forest, Cluj-Napoca (2014) and on trees vegetating near forests at Stana and Gălăşeni (2014), presumably disseminated by birds. Forest stands were generally mixed broadleaved forests dominated by *Quercus* species characteristic for hilly regions (Doniță et al., 1990).

Description of the pathogens

Both pathogens are currently placed in Basidiomycota, Exobasididiomycets, Microstromataceae after a relativelly long and old debate over their taxonomical position in Fungi Imperfecti or Basidiomycota (Wolf, 1927; Pires, 1928). They do not form blastic conidia, dispersing violently as other Exobasidiomycetidae. Basidia are of gastroid type, with well-developed sterigmata: in *Microstroma* spp., basidia develop in successive layers and protrude through stomata forming 2-6 basidiospores (Blanz, 1978; Begerow et al., 2001). Coelomycetous anamorph causes yellow spots of live leaves while the teleomorh emerges from stomata or disintegrated epidermis where it develops sori in which basidia are produced periodically. Basidiospores bud in a yeast like manner with subglobose to ellipsoid spores. The species present binucleate mycelium, with clamp connections (Begerow et al., 2001). Sterile hyphidia develop between basidia. Cultivation is defined by the formation of yeast like phase in haploid state, with subglobose to ellipsoid cells. The dimorphic morphology, with yeast stage and mycelial stage places the species in the large group of similar dimorphic organisms, around 700 recorded species placed in Basidiomycota and Ascomycota (Mösch, 2002).

Microstroma juglandis (Berenger) Sacc. develop necrotic areas containing the white frosty spots corresponding to basidiomata which are located along the midrib or randomly on the leaflet (Fig. 3). In heavy infections, the leaflets are deformed. The mycelium is endophytic but basidia are protruding throught the lower epidermis. Basidia are cylindrical to slightly clavate, of 18 x 9-10 µm with 6 sterigmata. Basidiospores are hyaline, oval shaped with pointed apices, of 5-8 x 2-3 µm (Ellis and Ellis, 1990).

Microstroma album (Desm.) Sacc. develop irregular yellow blotches on the upper leaf surface of *Quercus* spp. (Fig. 3) corresponding to frosty white spots on the lower surface (Fig. 3 shows the frosty white spots of *M. album* on *Q. robur, Q. petraea, Q.cerris* and *Q. pubescens* leaves). The pathogen is characterized by clustered basidia of 20-25 µm long bearing 4-6 sterigmata. The basidiospores are hyaline, ovate and slightly asymmetric, of 5-7 X 3 μm containing 2 guttules (Ellis and Ellis, 1990).

Molecular analysis showed that *Microstroma juglandis* is placed within the same clade with anamorphic *Rhodotorula phylloplana* which is a phylloplane species (Begerow et al., 2001) while *Microstroma album* clusters with *Rhodotorula baccarum*, both saprotrophic phyloplane species. The diseases are more frequent in rainy summers (Teviotdale et al., 2002).

Pathogenicity tests

Pure cultures of *Microstroma album* and *M. juglandis* were obtained on PDA (potato-dextrose-agar) medium. Isolation was performed from detached areas with lesions (for *M. album*, from *Q. petraea leaves* and for *M. juglandis*, from *Juglans regia* leaves), disinfected in 90% alcohol, repeatedly washed in sterile distilled water and placed on PDA medium supplemented with streptomycin. The cultures displayed a pale orange to pale brown coloration, wavy margins, areas of yeast like texture containing masses of spores and whitish cottony, sparse mycelium (Fig. 3).

Pathogenicity tests were performed on detached healthy leaves of *Quercus petraea* inoculated with a suspension of conidia from live culture of *M. album*. Detached leaves were placed to float on sterile water after inoculation and characteristic yellow spots appeared after one week of incubation at room temperature and under natural light regime. Same protocol of pathogenicity test was developed for *M. juglandis* using healthy *Juglans regia* leaves which confirmed the identity of pathogens.

Association of Microstroma album and Microstroma juglandis with other plant damaging species.

Aside the assessment of the presence of *Microstroma* species on hosts in different locations, the association with other species of pathogens or damaging arthropods was investigated. Since the most frequent associations were encountered in *M. album*, these were depicted in a network mode using the software Pajek 1.18 (Batagelj and Mrvar, 2010).

Network metrics applied to simplified configuration of pathogen-host-galling arthropods interaction were: connectivity, normalized node degree and betweeness centrality. Connectivity quantifies the proportion of realized links (L) in a network compared to all possible links as $2L/V(V-1)$ where V stands for vertices or nodes representing the interacting species, being a global network metric. As for centrality or importance metrics which give information on each vertex role or importance within the network, normalized node degree and betweeness centrality were employed.

The normalized node degree is the proportion of links connecting every node compared to all the links in the network quantifying the role or position of nodes within the network (Wasserman and Faust, 1994). This metric gives local information about a node (Jordán, 2005).

Betweeness centrality describes the importance of a vertex as a connector between different parts of a network (Freeman, 1979: Gonzáles et al., 2010)

$$BC_i = 2 \sum_{j<k;i\neq j} \frac{g_{ij}(i)/g_{jk}}{(n-1)(n-2)}$$

where: g_{jk} stands for the number of shortest path linking two species, $g_{jk}(i)$ is the number of those shortest path among g_{jk} that pass through vertex I and n is the number of species in the network. Species with BC>0 are considered connectors (Wasserman and Faust, 1994).

RESULTS AND DISCUSSIONS

According to field observations, seedlings of *Quercus* spp. were heavily infected, 75-80% of the foliage presenting the characteristic lesions, yellow leaf spots on the upper surface and white frosty deposits on the lower surface corresponding to basidia and masses of basidiospores. Trees vegetating within forest stands as well as isolated trees presented the symptoms.

Microstroma album was found in the aforementioned locations beginning with the summer of 2009.

Microstroma juglandis was found only in the summer of 2014 and 2013 and in more restricted areas. It is worth to mention that the summer of 2014 has been particularly rainy. It was found to associate frequently with *Marssonina juglandis* on the same leaves.

The species *Microstroma album* was found to associate on same leaves with several other species among which gall inducing insects, acari, and foliar pathogens. Less frequently leaves showed extended consumed areas due to free feeding insects and mines (mostly produced by *Tischeria ekebladella, Bacculatrix ulmella, Orchestes pillosus* or *Prophenusa pygmaea*).

Figure 2 depicts in graph mode, the relationship between *Microstroma album*, tree hosts and associated gall inducing insects (from genera *Neuroterus, Andricus, Cynips, Chilaspis*: Hymenoptera, Cynipidae, *Janetia*: Diptera, Cecidomyiidae) found in sampled areas, being the most frequently encountered pathogen-invertebrate interactions.

The connectivity of the network was low, only 13.68% of possible links across all species. Important vertices according to normalized degree values were: *Q. cerris*=0.52, *Q. robur, Q. petraea*=0.36 and *Microstroma album*= 0.21showing the densest linkage with neighbors within the network.

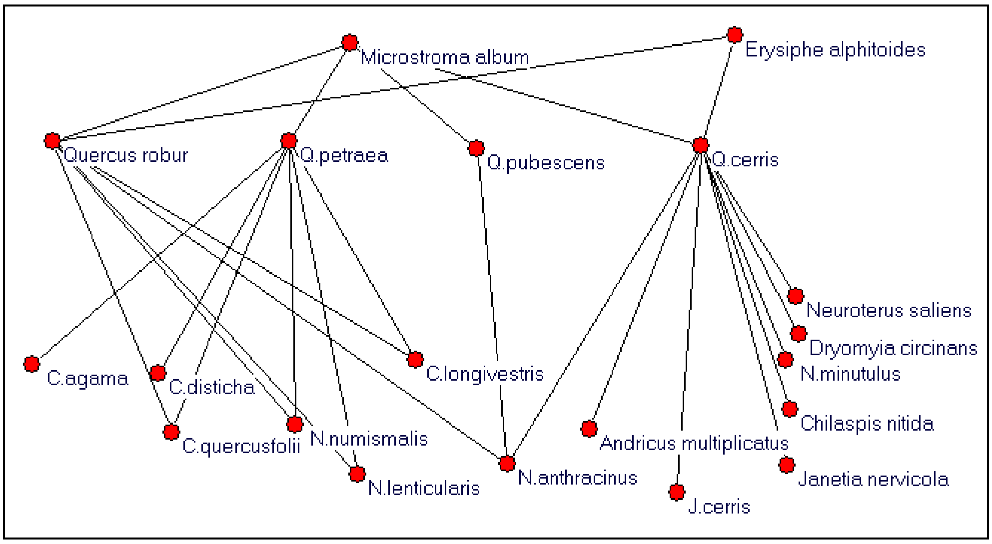

Fig. 2 Network of trithrophic interactions between tree hosts (*Q. robur, Q. petraea, Q. pubescens* and *Q. cerris*), obligate foliar pathogens (*Microstroma album* and *Erysiphe alphitoides*) and foliar galling insects (Pajek ver. 1.18)

In terms of connector species characterizing the network, highest values for betweeness centrality displayed *Q. cerris* (BC=0.63), *Microstroma album* (BC=0.32), *Q. petraea* (BC=0.28), *Q. robur* (BC=0.26). Surprisingly, *Erysiphe alphitoides* showed small BC (0.06), *Q. pubescens* even smaller (BC= 0.004) and most of specialized on one host galling insects had BC=0. Among galling insects, the most important player in terms of strategic advantage and importance within the guild showed *Neuroterus anthracinus* with BC=0.09.

Figure 4 presents the most frequently encountered galls associated with *M. album* on same host. The network is an undirected, qualitative graph containing 20 vertices and 26 edges, summarizing a trophic interaction among producers (*Quercus* spp. trees) and two categories of consumers, obligate foliar pathogens and galling insects.

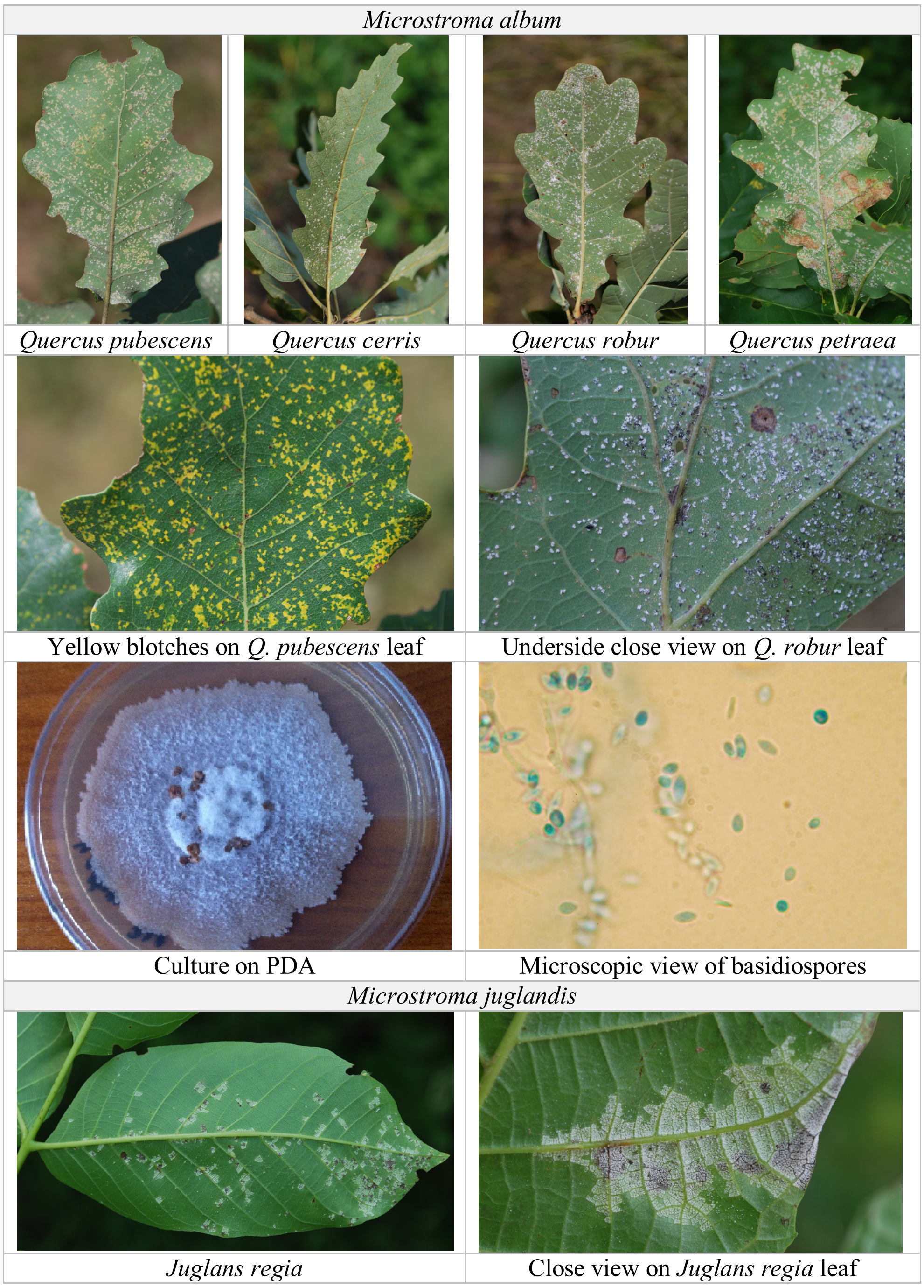

Fig. 3 White frosty deposits of *M. album* on *Quercus robur, Q. cerris, Q. petraea* and *Q. pubescens* leaves (first panel). Yellow blotches on upper side leaf and close view of the pathogen (second panel). Culture of *M. album* on PDA medium and microscopic image of basidiospores (third panel). *Microstroma juglandis* on *Juglans regia* leaves (fourth panel) (original photos)

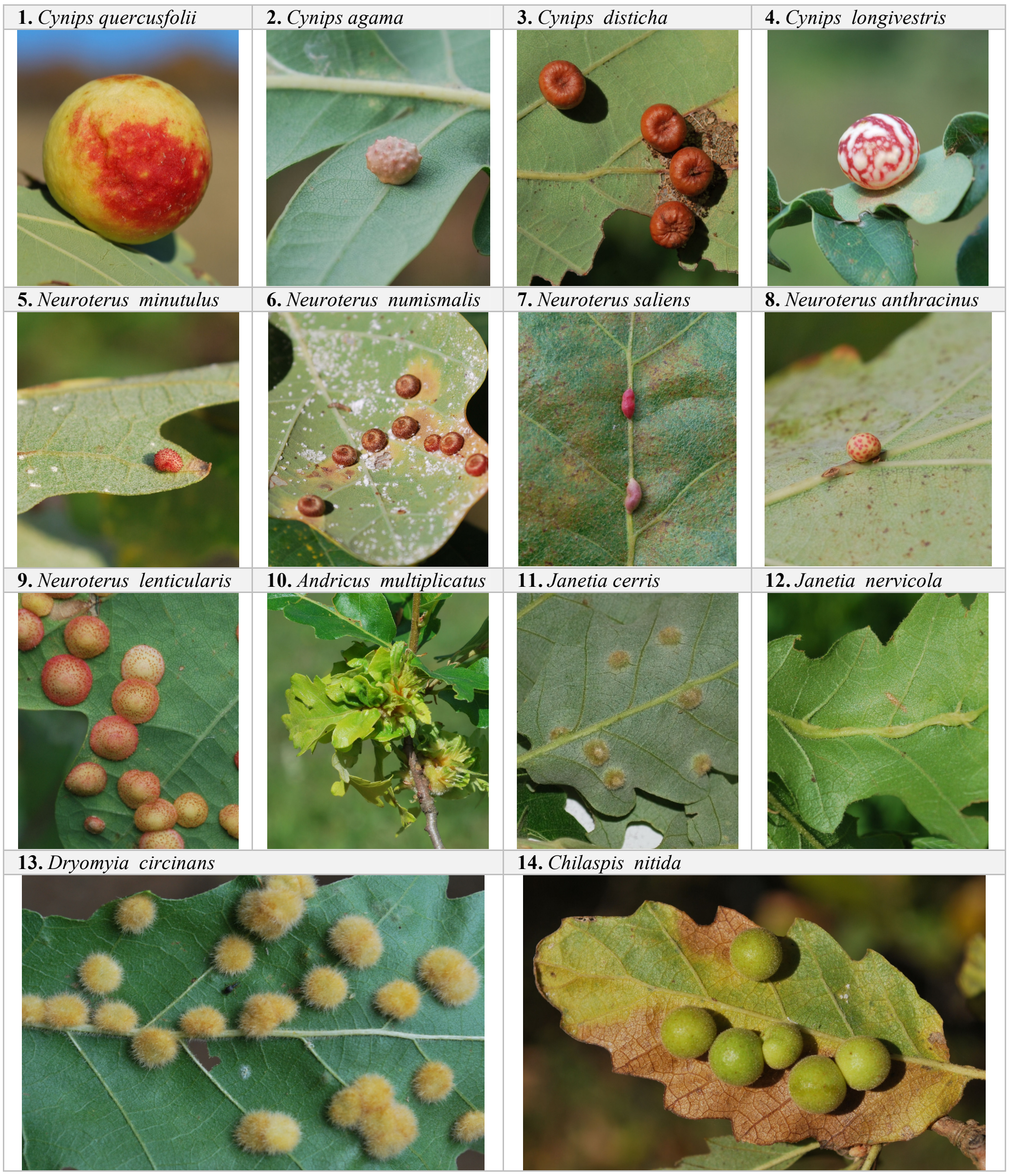

Fig. 4 Galls associated with *Microstroma album* and their host: *Q. robur* (1, 4, 8); *Q. petraea* (2, 3, 6, 9); *Q. cerris* (5, 7, 10-14) (original photos)

CONCLUSIONS

The fact that both *Microstroma* species evolved from a group of saprotrophically developing yeasts generally common on the leaves' surfaces characterized by ballistospores (spores discharged forcibly) is mostly interesting fact. Formerly rare species, with endemic spread, both species tend to become epidemic and our data confirm this trend.

It is noteworthy that in a relatively restricted geographic area of North-Western Romania, in mixed broadleaved forests harboring several species of *Quercus*, these have been extensively attacked by a previously infrequent obligate pathogen, *Microstroma album*.

The expansion during the last decades of *M. album* and *M. juglandis* must be understood in the frame of outbreak building diseases affecting natural forest stands and cultivated trees mediated by climate change and anthropogenic stress, a situation comparable with the expansion of another obligate foliar pathogen in Europe affecting oaks, *Erysiphe alphitoides*.

Under these circumstances, the interaction with other organisms using leaves for shelter and food appears to be inevitable. Moreover, the interaction network is an important variable in niche construction of plant pathogens (Fodor, 2009) which in turn, can be used in detection and modelling the spread of plant diseases (Meentemeyer et al., 2008). Our analysis showed that pathogens are important connectors (high node degrees) in networks established by trees and associated damaging organisms. Low connectivity of the proposed network reflects the narrow host specialization of galling arthropods however; hosts are linked by pathogens whose contribution to network metrics is consistent, especially of *Microstroma album*. Network analysis revealed that host trees were characterized by high node degrees and high BC values as a consequence of multiple consumers' guilds attacking a rich food resource, interconnected through hosts.

There are many groups of organisms which can induce galling, from insects to fungi, acari mites, viruses, bacteria and even parasitic plants. Aside their role in the complex network of forest biodiversity, galls produced by insects sustain a vast community of parasitoids and hiperparasitoid insects. Those represent the linkage and ensure the natural equilibrium between plants and phytophagous insects. In forest ecosystems galls are an important source of food for birds, predatory insects, other invertebrates and small mammals. They frequently associate with the tree pathogens and other damaging insects as the case of *M. album* indicated in our study. The most dissimilar in terms of associated gall inducing arthropods was *Quercus cerris* (Fig. 2) however, sharing same pathogens such as *Erysiphe alphitoides* and *Microstroma album* with other *Quercus* species vegetating in mixed broadleaved forests. The associated galling insects to *Q. cerris* were however highly specialized

Previously reported results on the interaction of *Erysiphe alphitoides* and guilds of foliivorous insects (galling, mining and free feeding insects) showed that there was differential outcome in terms of relative abundance of insects ranging from positive to negative (Tack et al., 2012). Our observations confirmed the interaction of foliar pathogens and foliivorous invertebrates using galling species as partners of interaction with *Microstroma album*, as this group is highly diversified and specialized on *Quercus* spp. (Abrahamson and Weis, 1987). It is most improbable that galling invertebrates interact directly with intercellular mycelium of *Microstroma album* since they develop their own separate structures relying on the diverted sap produced by the leaf. On the other hand, galling insects modify the metabolic pathways of the plant that in turn, exposes the plant to pathogen attack (Stone and Schönrogge, 2003) which in the case of *Quercus* species harboring a highly diversified guild of galling arthropods increases the chance of being exposed to foliar and shoot pathogens.

Our results confirm the fact that only sound ecological knowledge of the tree damaging guilds can ensure in the future a sustainable pest and pathogen management. Network formalism in the description of complex interactions between species associated to the same resource or host, niche modelling and knowledge of species life histories are few of theoretical tools which can be used in forest management activities in the future.

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