

Myxomycetes associated with a residential ecosystem

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Abstract

The myxomycetes associated with samples of the bark of living trees, ground litter, twigs, and aerial litter collected from a residential ecosystem were investigated with the use of moist chamber cultures. A total of 26 species representing 13 genera were recovered from 100 cultures prepared with samples of the four different types of substrates. The distribution patterns and ecology of these species are discussed in the context of the senior coauthor's more than 40 years studying myxomycetes.

Introduction

Myxomycetes, also called plasmodial slime molds or myxogastrids, are a group of amoeboid protists usually present and often abundant in terrestrial ecosystems, especially various types of forests (Stephenson and Stempen 1994). The myxomycete life cycle encompasses two very different trophic stages, one consisting of uninucleate amoebae, with or without flagella, and the other consisting of a distinctive multinucleate structure, the plasmodium (Martin and Alexopoulos 1969). Under favorable conditions, the plasmodium gives rise to one or more fruiting bodies (also referred to as sporocarps) containing spores. Although the fruiting bodies produced by myxomycetes are somewhat suggestive of those produced by higher fungi, they are considerably smaller (usually no more than 1–2 mm tall) and totally different in structure.

The fruiting bodies of myxomycetes develop in the field under natural conditions and are typically found in association with such substrates as decaying wood and various other types of dead plant material (Martin and Alexopoulos 1969). Fruiting bodies also appear on samples of dead organic material (especially pieces of the dead outer bark of living trees and dead leaves and other types of plant debris when these are collected, returned to the laboratory and used to prepare what are known as moist chamber cultures [Stephenson and Stempen 1994]). In both cases, the fruiting bodies can be collected and placed in small pasteboard boxes for permanent storage. If properly curated, fruiting bodies remain suitable for study for many years.

In the study reported herein, the myxomycetes associated with samples of the bark of living trees, ground litter, twigs, and aerial litter collected from a residential ecosystem were investigated with the use of moist chamber cultures. The study was prompted by the restrictions on travel and most normal activities imposed by COVID-19 during a major portion of 2020. The senior coauthor simply used the opportunity to carry out an investigation of the myxomycetes associated with the residential lot upon which his house is located.

Study area

The study area was a 90 by 120 foot (ca 27.4 by 36.6 m) residential lot (36°05'43" N, 94°07'47" W; elevation 420 m) located in a suburb of the city of Fayetteville, Arkansas. The center of the lot is occupied by a one-story, ranch-style house (Fig. 1), but the latter is surrounded by a total of 15 trees representing nine different species. These are apple (*Malus pumila* Mill.), box elder (*Acer negundo* L.), eastern red cedar (*Juniperus virginiana* L.), common persimmon (*Diospyros virginiana* L.), redbud (*Cercis canadensis* L.), shortleaf pine (*Pinus echinata* Mill.), silver maple (*Acer saccharinum* L.), sugar maple (*Acer saccharum* Marsch.), and tulip tree (*Liriodendron tulipifera* L.). Most of these trees occur as single individuals but there are four persimmons, two eastern red cedars and two sugar maples. In addition to the trees, several species of ornamental woody shrubs and herbaceous flowering plants are present on the lot, especially around the outside wall of the house.

Materials and methods

Samples of the dead outer bark of each of the different species of trees present on the lot represented the primary set of samples collected. In addition, samples of ground litter (including mulch that had been placed around some of the woody shrubs), twigs, and aerial litter (both tree leaves captured by the branches of the woody shrubs and aerial dead portions of the herbaceous flowering plants) also were collected.

All samples were returned to the laboratory (in this case a small laboratory in the senior coauthor's house) and used to prepare a series of moist chamber cultures in the manner described by Stephenson and Stempen (1994). Moist chamber cultures consisted of 90 mm plastic disposable Petri dishes lined with filter paper. Five moist chambers were prepared from each 20 different types of samples.

The entire set of samples consisted of (1) hardwood twigs [mostly from persimmon], (2) leaf litter collected from beneath ornamental shrubs, (3) bark from eastern red cedar, (4) bark from apple, (5) bark

from persimmon, (6) bark from pine, (7) pine twigs, (8) bark from eastern redbud, (9) bark from sugar maple, (10) bark from box elder, (11) bark from silver maple, (12) mulch from around ornamental shrubs, (13) leaf litter collected from beneath ornamental shrubs [front of house], (14) leaf litter collected from beneath ornamental shrubs [back of house], (15) bark from tulip tree, (16) aerial litter of a liliaceous ornamental plant, (17) pine needles, (18) aerial litter of an asteraceous herbaceous ornamental plant, (19) aerial litter consisting of tree leaves trapped in branches of a shrub, and (20) aerial litter from a semi-woody shrub. The mulch was the only substrate that had been introduced from outside the study area. Bags of mulch had been purchased from a local store a couple of years previously and then added to the ground around the shrubs. It consisted of intermixed pieces of shredded wood and bark and was well weathered when collected as part of this study.

Moist chamber cultures were monitored over a period of approximately four months. Water was added as necessary to keep the cultures from drying out. When the fruiting bodies of myxomycetes were observed, they were recorded. In some instances, sporocarps and particularly spores were prepared as semipermanent slides in polyvinylalcohol to observe microscopic features. Standard references (e.g., Martin and Alexopoulos 1969, Ing 1999, Poulain et al. 2011) were used to identify fruiting bodies to species.

Results

The 100 moist chamber cultures yielded 26 species of myxomycetes in 13 genera. Eighty percent of all cultures produced some evidence (either fruiting bodies or plasmodia) of myxomycetes. Fruiting bodies were recorded in 60 of the positive cultures and only plasmodia appeared in 20 cultures. There were 119 records of fruiting bodies, 116 of which could be identified to the level of species. Three records consisted of immature or aberrant material and were not possible to identify. The mean number of species recorded from those cultures with fruiting bodies present was 1.58. The highest number of species recorded from a single moist chamber culture

was five. Eastern red cedar bark was the single most productive substrate, with the five cultures yielding eight species, but six species were recovered from the set of five cultures for four other substrates (box elder bark, sugar maple bark, silver maple bark, and leaf litter from a semi-woody shrub). In contrast, no fruiting bodies appeared on samples from hardwood twigs, mulch, trapped tree leaves, and aerial litter from a semi-woody shrub, although only the trapped tree leaves did not produce at least one plasmodium.

Annotated list of species

All species of myxomycetes recorded in the present study are listed alphabetically by genus and then species. The nomenclature used follows Lado (2005–2021). Information is provided on the total number of collections and the substrate(s) yielding the species in question, with the numbers corresponding to those used in the list of substrates given in the section on Materials and Methods.

Arcyria cinerea (Bull.) Pers.

Substrates 3, 4, 7, 11, 13, 14, 16 and 19 (17 records)

Arcyria marginoundulata Nann.-Bremek. & Y. Yamam.

Substrates 13 and 14 (five records)

Calomyxa metallica (Berk.) Nieuwl.

Substrates 8, 9 and 10 (four records)

Badhamia cf. *nitens* Berk. **Fig. 2**

Substrates 3, 4 and 5 (ten records)

Clastoderma debaryanum A. Blytt

Substrate 9 (one record)

Comatricha nigra (Pers. ex J.F. Gmel.) J. Schröt.

Substrate 7 (one record)

Cribraria violacea Rex

Substrates 3, 4, 5 and 10 (14 total records)

Diderma chondrioderma (de Bary & Rostaf.) Kuntze

Substrates 8 and 9 (two records)

Diderma effusum (Schwein.) Morgan

Substrate 18 (two records)

Diderma hemisphaericum (Bull.) Hornem.

Substrate 2 (two records)

Didymium clavus (Ab. & Schwein.) Rabenh.

Substrate 9 (one record)



Figure 1. The study area where the samples used in the present study were collected.

Didymium diffforme (Pers.) Gray

Substrate 18 (two records)

Didymium ochroideum G. Lister

Substrate 13 (three records)

Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist

Substrates 7 and 13 (two records)

Echinostelium minutum de Bary

Substrate 7 (one record)

Licea biforis Morgan

Substrate 18 (one record)

Licea parasitica (Zukal) G. W. Martin

Substrates 8, 10 and 11 (nine records)

Licea rugosa Nann.-Bremek. & Y. Yamam.

Substrate 10 (one record)

Perichaena chrysosperma (Curr.) Lister

Substrates 3, 9, 10, 13 and 16 (five records)

Perichaena depressa Lib.

Substrates 13 and 15 (three records)

Perichaena vermicularis (Schwein.) Rostaf.

Substrates 3, 4 and 9 (nine records)

Physarum cinereum (Batsch) Pers.

Substrates 16 and 18 (eight records)

Physarum crateriforme Petch

Substrate 10 (six records)

Physarum melleum (Berk. & Broome) Massee

Substrate 11 (three records)

Physarum pusillum (Berk. & M.A. Curtis) G. Lister

Substrate 17 (two total records)

Stemonitis herbatica Peck

Substrate 7 (two total records)

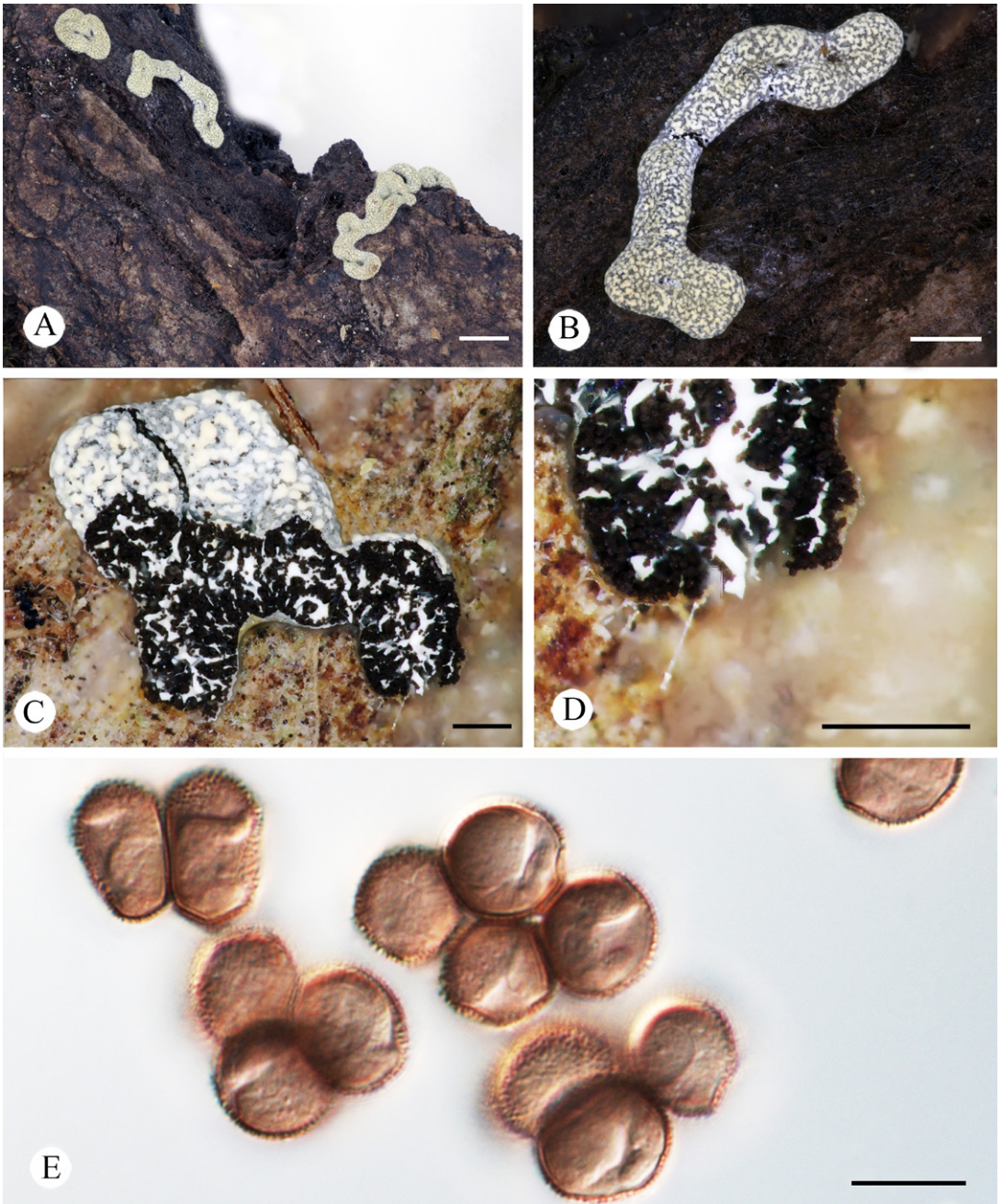


Figure 2. Morphological features of the specimens identified as *Badhamia cf. nitens* (SLS 34401) in the present study. **A:** plasmodiocarps as observed under a dissecting microscope (DM), **B:** plasmodiocarp (DM), **C:** opened plasmodiocarp (DM), **D:** capillitium and spore mass (DM), **E:** Spores as observed with a light microscope (LM) and differential interference contrast (DIC, 100x). Scale bars: A = 1000 μm , B = 500 μm , C, D = 200 μm , E = 10 μm .

Discussion

There have been relatively few studies of myxomycetes in urban settings (Wrigley de Basanta 2000, Chen et al. 2005, Rincón-Marín et al. 2021). The most comparable study of which we are aware was carried by Hosokwa et al. (2019), who examined the myxomycetes appearing in moist chamber cultures prepared with samples collected in 32 inner-city and semi-urban parks in Sydney, Australia. These authors recorded 26 species that they could identify to species along with at least half a dozen others that were identified only to genus. The substrates they used to prepare moist chamber cultures were ground litter, dead twigs, and bark. The number of species reported in their study was the same as the total 26 taxa reported in the present study, although the total area sampled in Australia was considerably larger. Only five of the species reported by Hosokwa et al. (2019) were also recorded in the present study.

Tucker et al. (2011) carried out a study of the myxomycetes associated with the dead leaves and stems of house plants (i.e., potted ornamental plants growing inside of a house) in the city of Fayetteville. They recovered 12 species, including five also recorded in the present study. All of the other studies carried out in Arkansas have considered the myxomycetes associated with substrates collected from nature but not in an urban setting. The records of myxomycetes reported were based on specimens collected directly in the field or appearing in moist chamber cultures. These other studies include Eliasson et al. (1988), Goad and Stephenson (2013), Massingill and Stephenson (2013), Fischer and Stephenson (2014), Clayton et al. (2014), dela Cruz et al. (2014), and Stephenson et al. (2020). As result, the myxomycetes of Arkansas are relatively well known. Based on the data reported in the papers mentioned above, with a single exception all of the species of myxomycetes recorded in the present study are common enough so that their occurrence in a residential lot would not be unexpected. The one exception was *Badhamia* cf. *nitens*, which had not been reported previously from Arkansas. This species is known from scattered localities throughout the world but does not appear to be particularly common.

Badhamia nitens is superficially similar to certain species in the genus *Physarum* that typically

have yellow lime on the peridium and characteristically produce sporocarps that are sporangia or short plasmodiocarps, including *P. lakhanpalii* Nann.-Bremek. & Y. Yamam. Both *B. nitens* and *P. lakhanpalii* have spores that adhere in clusters. Initially, specimens recovered in the present study were referred to *P. lakhanpalii*, but several examples were sent to the laboratory of the second coauthor at the Komarov Botanical Institute. These were sequenced and found to be a better fit for *B. nitens*, based on a consideration of microscopic features of the fruiting body (Fig. 2) and comparisons with the few other sequences available for this species. However, the specimens reported herein are currently being subjected to more detailed study, and a final decision on their taxonomic position will be made after the phylogenetic relationship of what is currently recognized as *B. nitens* evaluated more completely once additional material of this apparently rare species can be obtained.

Interestingly, the specimens referred to *B. nitens* in the present study seem to represent two different forms, one in which the spores adhered in tight clusters and the other in which the spores were in loose clusters that readily break apart. Although this might suggest the possibility that two species are involved, in one instance both forms were recorded on the same substrate (persimmon bark).

Stephenson (1988), in a study carried out in the upland forests of southwestern Virginia, was able to apply the concept of niche to the more abundant species of myxomycetes he recorded from five different study areas that differed in microclimate and vegetation. He demonstrated that different species tended to display recognizable patterns of distribution with respect to the gradients he defined. As might have been expected, based on other studies of niche, some species had broad niches while for others the niche was narrow. If the 20 types of substrates examined in the present study are considered to represent a single gradient, then the number of different substrates on which a species was recovered would, in essence, represent its niche breadth. On this basis, species such as *Arcyria cinerea* (eight different types of substrates) and *Perichaena chryosperma* (five different types of substrates) would be considered to have broad niches, whereas those species recorded from only a single type of substrate (e.g., *Physarum pusil-*

lum and *Licea biforis*) would be considered to have a narrow niche. Obviously, there are factors other than substrate that determine the distribution of myxomycetes, but the patterns reflected in the data presented herein would seem to provide an insight into this aspect of myxomycete ecology.

A second niche axis that could be considered in the present study was the one associated with time of development. Some species developed early in the period of observation while others took much longer. The first species recorded (*Comatricha nigra*) appeared after only one week and presumably developed from a sclerotium already present on the substrate. Next to develop were such species as *Arcyria cinerea*, *Calomyxa metallica*, and *Badhamia* cf. *nitens*. These were followed by a group of species that included *Cribraria violacea*, *Echinostelium minutum*, and *Perichaena vermicularis*. The last group to appear consisted of such species as *Physarum pusillum*, *Perichaena chryosperma*, and *Physarum melleum*. Interestingly, two species (*Cribraria violacea* and *Arcyria cinerea*) produced fruiting bodies over appreciable periods of time, approximately six weeks for *A. cinerea* and eight weeks for *C. violacea*.

It has long been known that various substrates are not equally productive for myxomycetes (Martin and Alexopoulos 1969). The patterns of occurrence of corticolous (bark-inhabiting) myxomycetes have been studied the most, and it is clearly apparent that the bark of some types of trees is especially favorable for these organisms. This has been reported to be the case for eastern red cedar (e.g., Keller and Brooks 1973), so the fact that eight different species were recorded from this substrate is probably not surprising.

Since the spores of myxomycetes are thought to be distributed mostly by wind (Stephenson and Stempen 1994), the physical features of some substrates make them better "spore traps" than others (Takahashi 2014). As noted earlier, aerial litter consisting of tree leaves trapped in branches of a shrub was the one substrate that did not yield any evidence of myxomycetes. The surfaces of these leaves were smooth and not conducive to trapping spores from the air. In contrast, several of the other substrates were characterized by rough surfaces that are likely to be much more effective spore traps.

Although the study reported herein was limited

in scope, the results clearly indicated that any survey for myxomycetes should incorporate as many different types of substrates as possible in an effort to make it as comprehensive as possible. The twenty different substrates investigated do not represent the full range of substrates (or microhabitats, as the term is often used for myxomycetes (e.g., Stephenson et al. 2020) present in the study area being considered. The soil microhabitat and the ground litter microhabitat associated with the grassy lawn are two examples that undoubtedly would have yielded additional species.

It should be noted that the research project described herein was relatively simple and carried out with very little in the way of special equipment other than two microscopes (one compound and the other dissecting). As such, it serves as an example of the type of project that could be carried out by amateur mycologists as well as students from a wide range of grade levels.

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