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Myxomycetes associated with the aerial litter microhabitat in a temperate deciduous forest

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Abstract: The myxomycetes associated with the aerial litter microhabitat were investigated in a temperate deciduous forest in southwestern Virginia, USA. Samples of aerial litter were collected from herbaceous and woody plants and used to prepare a series of 48 moist chamber cultures. Sixty-five percent of these yielded evidence (plasmodia and/or fruiting bodies) of myxomycetes. Eleven species in nine genera were recorded, but *Diderma effusum*, *Physarum cinereum*, and *Arcyria cinerea* made up 66% of all specimens. Samples from herbaceous plants were relatively more productive (78% positive cultures, eight species) than those from woody plants (60% cultures, six species).

Keywords: ecology, mixed oak forest, moist chamber cultures, slime molds, southwestern Virginia

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 et al. 1983). 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 certain 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 substrates such as coarse woody debris and various other types of dead plant material (Martin and Alexopoulos 1969). Fruiting bodies also appear on samples of dead plant material 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.

Aerial litter has been defined as dead but still attached plant parts that have never been in contact with the ground (Stephenson and Spooner 1997, Black et al. 2004, Stephenson et al. 2004). In tropical forests, this microhabitat includes the dead leaves of epiphytes, dead aerial portions of both herbaceous and woody plants, and dead leaves trapped by such things as lianas and aerial rhizomorphs (Stephenson 2020). Aerial litter is often abundant in tropical forests but relatively uncommon in temperate forests, especially during the summer. However, scattered examples do exist (Figure 1), although the first co-author is not aware that they have ever been investigated in temperate forests during summer to determine if they do support myxomycetes.

Figure 1. Example of aerial litter in a mixed oak forest in southwestern Virginia.

The purposes of the study reported herein were (1) to determine if myxomycetes are associated with aerial litter in a temperate deciduous forest during the summer, (2) to characterize the assemblage of species present if myxomycetes do occur in this microhabitat, and (3) to assess the differences in the species associated with the aerial litter of herbaceous plants and aerial litter of woody plants.

Materials and methods

On May 30 and June 1 of 2021, twenty samples of aerial litter (consisting of dead leaves in each instance) were collected in the general vicinity of the University of Virginia Mountain Lake Biological

Station (38.0342°N, -78.5120°W; elevation 1160 m) in southwestern Virginia. The forest from which samples were collected is mixed oak, with northern red oak (*Quercus rubra* L.) and white oak (*Q. alba* L.) as the dominant species present. Six of the samples consisted of leaves collected from herbaceous plants, whereas the remaining fourteen samples consisted of dead leaves from woody plants.

Herbaceous plants providing dead leaves were wild geranium (*Geranium maculatum* L.), intermediate shield fern (*Dryopteris intermedia* [Muhl. ex. Willd.] A. Gray), sweet-cicely (*Ozmorhiza longistylis* [Torr.] DC.), an unidentified wood aster (*Aster* sp.), and broad-leaved panic grass (*Dichanthelium latifolium* [L.] Harvill. Woody plants sampled were great laurel (*Rhododendron maximum* L.), red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), witch hazel (*Hamamelis virginiana* L.), common serviceberry (*Amelanchier arborea* [F. Michx.] Fernald), and an unidentified willow (*Salix* sp.).

Each sample was placed in a small paper bag in the field, brought back to the Eumycetozoan Laboratory at the University of Arkansas, and processed in the manner described by Stephenson and Stempen (1994). In brief, pieces of dead leaves from each sample were placed in disposable plastic Petri dishes lined with filter paper, with enough material placed in each dish to cover most of the bottom. Later, water was added to each dish, the top placed on the dish, and what was now a moist chamber culture was set aside for approximately 24 hours. After this time, pH was determined for each culture with the use of a portable pH meter and then excess water was poured off. One to four moist chamber cultures were prepared from each sample, for a total of 48 cultures (13 from herbaceous plants and 35 from woody plants). These cultures were examined with a stereomicroscope on a weekly basis for a period of three months. Specimens of myxomycetes appearing in the cultures were removed along with a small portion of the substrate upon which they occurred, allowed to air-dry, and then placed in small pasteboard boxes for permanent storage. Identifications were made by the first co-author, based on his more than 40 years working with myxomycetes. All specimens cited herein are now deposited in the mycological herbarium (UARK) of the University of Arkansas.

Results

Thirty-one (65%) of the 48 moist chamber cultures prepared with samples of aerial litter yielded evidence (either fruiting bodies or plasmodia) of myxomycetes. Samples of herbaceous aerial litter were relatively more productive (78%) than those of woody litter (60%) when numbers of positive cultures were considered in spite of the higher number of moist chamber cultures prepared for woody plants. Eleven species in nine genera were recorded. *Diderma effusum* (specimens appearing in ten cultures), *Physarum cinereum* (seven cultures), and *Arcyria cinerea* (seven cultures) were the most common species. Collectively, these three species represented 66% of all specimens. Herbaceous aerial litter yielded 17 specimens, whereas woody aerial litter yielded 18 specimens. However, cultures prepared with herbaceous aerial litter were far more productive when the mean number of species appearing in each moist chamber culture was considered (1.2 species per culture for herbaceous aerial litter and only 0.5 species per culture for woody aerial litter).

Most of the myxomycetes appearing in the moist chamber cultures were represented by only a few fruiting bodies, but this was not always the case. One of the cultures prepared with a sample of woody aerial litter produced more than 200 fruiting bodies of *Arcyria cinerea*.

The mean pH of all moist chamber cultures was 6.7 (range = 4.6 to 7.5). The mean pH value for cultures prepared with herbaceous aerial litter was 6.8 (range = 4.8 to 7.5), which was slightly higher than the value recorded for woody litter (6.4, with a range of 4.6 to 7.0).

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 (herbaceous and/or woody aerial litter) yielding the species in question. Collection numbers are those of the first co-author.

Arcyria cinerea (Bull.) Pers.
Herbaceous aerial litter (three records, including 34489) and woody aerial litter (four records, 34541)

Cribraria microcarpa (Schrad.) Pers.
Woody aerial litter (one record, 34472)

Diderma effusum (Schwein.) Morgan
Woody aerial litter (eight records, including 34438 and 34486) and herbaceous aerial litter (two records, including 34495)

Didymium difforme (Pers.) Gray
Herbaceous aerial litter (two records, including 34435)

Didymium squamulosum (Alb. & Schwein.) Fr. & Palmquist
Herbaceous aerial litter (one record, 34497)

Lamproderma scintillans (Berk, & Broome) Morgan
Woody aerial litter (two records, including 34488)

Licea biforis Morgan
Herbaceous aerial litter (one record, 34436B)

Perichaena vermicularis (Schwein.) Rostaf.
Herbaceous aerial litter (two records, including 34445)

Physarum cinereum (Batsch) Pers.
Herbaceous aerial litter (five records, including 34489 and 34498) and woody aerial litter (two records, including 34498)

Physarum viride (Bull.) Pers.
Herbaceous aerial litter (one record, 34563)

Stemonitis flavogenita E. Jahn
Woody aerial litter (one record, 34599)

Discussion

The data obtained in the present study indicate that there is an assemblage of myxomycetes associated with the aerial litter microhabitat in temperate deciduous forests during the period of the year when a closed canopy is present. This microhabitat is not well-developed in these forests, as indicated by

the time and effort required to collect the twenty samples considered herein. This was particularly true for examples of aerial litter from herbaceous plants. However, this type of aerial litter was easily accessible where it did occur. Examples of aerial litter from woody plants (mostly trees) were typically far enough above the ground that they were impossible to collect.

It is not surprising that many of the species appearing on aerial litter were listed as associated with "dead leaves…on leaves…plant remains…leaves" by Martin and Alexopoulos (1969). The usual substrate for the one exception (*Cribraria microcarpa*) was indicated as dead wood, but in the experience of the first co-author, this species can occur on leaves.

The low numbers of records for the majority of species recorded in the present study do not allow ecological differences in the two types of litter to be examined. Nevertheless, it is interesting that eight of the ten records of *Diderma effusum* appeared on woody aerial litter, which suggests an affinity of this species for this type of litter.

In brief, the present study was limited in scope, and the most important contribution of the research carried out is that it sets the stage for a more extensive investigation of a microhabitat that has only rarely been considered. For example, this was the case for the previous major study of the distribution and ecology of myxomycetes carried out in the Mountain Lake area by the first coauthor (Stephenson 1988, 1989). Although the aerial litter microhabitat in temperate deciduous forests is rather limited in extent, it does support an assemblage of myxomycetes and thus should be regarded as a minor component of this type of ecosystem.

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