Mycosphere 4 (4): 707–712 (2013)
www.mycosphere.org
Copyright © 2013

Article

ISSN 2077 7019
Mycosphere
Online Edition

Doi 10.5943/mycosphere/4/4/7

Myxomycetes appearing in moist chamber cultures on four different types of dead leaves

Goad AE[1] and Stephenson SL[1,*]

[1]*Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701*

Goad AE,Stephenson SL 2013 – Myxomycetes appearing in moist chamber cultures on four different types of dead leaves.Mycosphere 4(4), 707–712, Doi 10.5943/mycosphere/4/4/7

Abstract

One of the microhabitats available for myxomycetes in temperate forests is represented by the layer of dead plant material that accumulates on the forest floor. This layer, referred to as the litter layer, consists mostly of dead leaves from the trees that make up the forest canopy. In the present study, leaves from four different species of trees (dogwood, red maple, sycamore and white oak) were collected from the upper portion of the litter layer and used to prepare moist chamber cultures to determine whether each type of leaf displayed evidence of supporting a different ecological assemblage of myxomycetes. Leaves were collected on two occasions, January 2012 and January 2013. Forty moist chamber cultures were prepared for each type of leaf on each date, for a total of 320 cultures for the entire study. Most of these (60.6%) yielded some evidence (either sporocarps or plasmodia) of myxomycetes, and 21 species in nine genera were represented among the 244 records of sporocarps recorded from the cultures. Members of the order Physarales made up 59% of all records, with members of the orders Stemonitales (30%) and Trichiales (11%) relatively less important. Major differences existed in the productivity of the two sets of leaf samples (with 2013 appreciably more productive than 2012) and for particular types of leaves (with dogwood characterized by an overall positive value of 92.5% and sycamore with an overall positive value of only 11.5%). Remarkably, the set of samples of sycamore leaves collected in 2013 yielded no positive cultures.

Key words – Devil's Den State Park – ecological study – litter layer – slime moulds

Introduction

The myxomycetes (also referred to as myxogastrids or plasmodial slime moulds) are a group of fungus-like organisms that have been known from their fruiting bodies (or sporocarps) since at least the mid-17th century (Martin & Alexopoulos 1969, Stephenson 2011). Approximately 900 species are known (Lado 2005-2013), and some of these apparently occur anywhere on earth where there are plants and thus plant detritus. Myxomycetes are often common in nature, where their sporocarps are found associated with such microhabitats as decaying coarse woody debris and forest floor leaf litter. However, most species are inconspicuous or sporadic in their occurrence and thus often difficult to detect in the field. As such, the moist chamber culture technique as it applies to myxomycetes (Gilbert & Martin 1933) provides a convenient and often very productive method of supplementing field collections when studying such microhabitats as forest floor leaf litter (Stephenson 1989).

Submitted 22 May 2013, Accepted 17 July 2013, Published online 30 July 2013
*Corresponding author: Steven L. Stephenson– e-mail – slsteph@uark.edu

In temperate forests, myxomycetes are known to occur on the dead leaves that make up a major portion of the litter layer that occurs on the forest floor (Stephenson 1988, Stephenson & Stempen 1994). Except in those exceptional instances in which a forest is dominated by a single tree species, the litter layer is relatively heterogeneous, consisting of an often complex mixture of dead leaves derived from a number of different species of trees and other plants. In addition to the dead leaves, the litter layer also contains other types of plant material, including small pieces of dead bark, woody twigs and various plant-derived reproductive structures (e.g., seeds, fruits, inflorescences and cones).

The leaves of most trees are distinctive enough to be identified rather easily after they have fallen to the forest floor, so it is possible to collect a series of leaves derived from the same species of tree so long as the leaves are still reasonably intact. In the present study, leaves from four different species of trees were collected from the upper portion of the litter layer and used to prepare moist chamber cultures to determine whether each type displayed evidence of supporting a different ecological assemblage of myxomycetes. All leaves were collected at the same locality.

Materials & Methods

Samples of dead leaves of dogwood (*Cornus florida* L.), red maple (*Acer rubrum* L.), sycamore (*Platanus occidentalis* L.), white oak (*Quercus alba* L.) and red maple (*Acer rubrum* L.) were collected from Devil's Den State Park in northwest Arkansas (35° 46' N, 94° 14' W; elevation ca 390 m), located south of the city of Fayetteville. Leaf samples were collected on two different occasions, the first in January 2012 and the second in January 2013. All of the samples were returned to the Eumycetozoan Laboratory at the University of Arkansas, where they were used to prepare a series of 40 moist chamber cultures for each leaf type in each of the two years, for a total of 320 cultures for the entire study. The culture chambers used consisted of 15 cm plastic disposable Petri dishes, each lined with piece of filter paper. Enough sample material was placed in the dish to cover the bottom as completely as possible yet still allowing a lid to be placed securely on the dish. Distilled water was then added to each Petri dish and the latter left undisturbed for a period of approximately 24 hours, when the pH of what had become a moist chamber culture was determined with a portable pH meter and a flat surface electrode. After the value of pH had been recorded, most of the water in each Petri dish was poured off, and the moist chamber cultures stacked and placed in an area of the laboratory receiving only diffuse light. Each culture was examined once a week for several months, and water was added when necessary to maintain moist conditions. When the sporocarps of myxomycetes appeared in a culture, the piece (or pieces) of leaf upon which they occurred was removed, glued to a small paper tray, and the latter mounted in a pasteboard box for permanent storage.All occurrences of the same species in a single moist chamber culture were considered to represent one record. Identifications were made with the use of standard monographs. Nomenclature used herein essentially follows Lado (2005-2013) except for *Stemonitis nigrescens*, where the treatment used is that of Martin & Alexopoulos (1969). Voucher specimens of all species are deposited in the herbarium of the University of Arkansas (UARK).

The sets of data obtained for the different types of leaves were compared using coefficient of community and percentage similarity indices (Mueller-Dombois & Ellenberg 1974, Gauch 1982). The equation for the former, which is based solely on a consideration of the presence or absence of species, is coefficient of community (CC) = 2c/(a + b) where *a* is the total number of species in the first community (or assemblage) being considered, *b* is the total number of species in the second community, and *c* is the number of species shared in common. The value of CC ranges from 0 (when no species are present in both communities) to 1.0 when all species are present in both communities.

The equation for percentage similarity, which considers the relative abundance of species in a community and not just their presence, is percentage similarity (PS) = Σ min (a, b, …x) where *min* is the lesser of the two percentage composition values of species *a, b, ….x* in the two communities. The value of PS ranges from 0 for communities with no species in common to 1.0 for communities identical both in species composition and quantitative values for the species.

In addition, species diversity indices were calculated for the sets of data using Shannon's formula (Shannon & Weaver 1963), which is species diversity (H') = -Σ Pi log Pi where *Pi* is the proportion of the total number of individuals represented by species *i*. This index varies from a value of 0 for a community containing a single species to some maximum value for a community containing many species, each represented by a small number of individuals.

In order to assess differences in levels of various nutrients that might exist for the four types of leaves, some of the sample material was ground into a power, mixed thoroughly and sent to Brookside Laboratories (New Knoxville, Ohio) for analysis of nitrogen, phosphorus, magnesium, potassium, calcium, sulfur, boron, iron, manganese, copper, zinc and aluminum.

Results

The majority (60.6%) of the 320 moist chamber cultures yielded some evidence (either sporocarps or plasmodia) of myxomycetes, and 21 species in nine genera were represented among the 244 records of sporocarps collected from these cultures (Table 1). Three collections (*Comatricha* sp. A, *Comatricha* sp. B, and *Physarum* sp. A) could be identified only to genus because of the very limited material available (only a single sporocarp in each instance). However, each was clearly distinct from all other species in the same genus recorded during the present study. Members of the order Physarales made up 59% of all records, with members of the orders Stemonitales (30%) and Trichiales (11%) relatively less important.

Major differences existed in the productivity of the two sets of leaf samples and for particular types of leaves. Samples collected in 2012 yielded 69 records and 12 species, whereas those collected in 2013 produced 175 records and 16 species. Only seven species (*Arcyria cinerea*, *Didermaeffusum*, *D. hemisphaericum*, *D. ochroideum*, *D. testaceum*, *Lamproderma scintillans*and *Physarum bivalve*) were recorded in both years. Several species were common to abundant one year and uncommon or even absent the other year. Prominent examples were *Physarum serpula* (21 records in 2012 and none in 2013), *Diderma effusum* (67 records in 2013 and only one record in 2012), *D. hemisphaericum* (27 records in 2013 and just two records in 2012) and *Perichaena chrysosperma* (22 records in 2013 and none in 2012). Indeed, *Lamproderma scintillans* was the only species represented by >20 records in both years. Dogwood leaves were the most productive, with an overall positive value of 92.5% for pooled data for both years, whereas sycamore was the least productive, with a overall positive value of only 11.5%. Values for red maple and white oak were 71.3% and 67.5%, respectively. The productively of sycamore was extraordinary low, and no sporocarps ever developed in moist chamber cultures prepared with the leaves of this tree. Indeed, the set of samples of sycamore collected in 2013 yielded no positive cultures (i.e., not even evidence of plasmodia). In marked contrast, 50 of the 69 (72%) collections recorded in 2012 and 102 of the 175 (58%) recorded in 2013 appeared on dogwood.

Values of pH recorded for moist chamber cultures and results obtained for nutrient levels in the four types of leaves are provided in Tables 2 and 3. These data indicate that pH can vary rather widely among different types of leaves, with a range of 4.0 to 7.1 being recorded in the present study. White oak and red maple were relatively more acidic than dogwood or sycamore, and there was no overlap between the two groups. Although this general pattern held true for samples collected in the two years, the values obtained for a particular type of leaf displayed some noticeable differences. For example, red maple leaves collected in 2013 were less acidic than those collected in 2012. Nutrient levels varied from one type of leaf to another, but no single type was characterized by values that were clearly different from the other types when samples from both years are considered. One of the possible exceptions was red maple, which had noticeably higher levels of manganese in 2013. As a general observation, values recorded for leaf samples collected in 2013 were higher than for samples collected in 2012. In some instances (e.g., levels of potassium in all four types of leaves but especially for white oak, red maple and sycamore), leaves collected in 2013 were characterized by appreciably higher values. Especially noteworthy are the exceedingly high values of iron and aluminum recorded for dogwood. Although based on observations made while the moist chamber cultures were being maintained, sycamore leaves tended to dry out

Table 1 Occurrence of myxomycetes on the four different types of leaves. Numbers of records from samples collected in 2012 are given in parentheses, whereas those outside the parentheses are records from samples collected in 2013.Note: DW = dogwood, WO = white oak, RM = red mapleand SY = sycamore.

Species	Total	DW	WO	RM	SY
Diderma effusum (Schwein.) Morgan	68	17	18	32 (1)	
Lamproderma scintillans (Berk. & Broome) Morgan	59	32 (22)		(5)	
Diderma hemisphaericum (Bull.) Hornem.	29	25 (2)		2	
Perichaena chrysosperma (Curr.) Lister	22	22			
Physarum serpula Morgan	21	(19)	(2)		
Physarum bivalve Pers.	10	(3)	(3)	1 (3)	
Stemonitis herbatica Peck	6		6		
Didymium ochroideum G. Lister	6	5 (1)			
Comatricha pulchella (C. Bab.) Rostaf.	4		4		
Stemonitis nigrescens Rex	3		3		
Arcyria cinerea (Bull.) Pers.	3		1 (1)	1	
Diderma cf. *minutum* Nann.-Bremek.	2	(2)			
Diderma testaceum (Schrad.) Pers.	2			1 (1)	
Didymium iridis (Ditmar) Fr.	2		(1)	(1)	
Comatricha sp. A	1		1		
Comatricha sp. B	1		1		
Didymium cf. *anellus* Morgan	1	1			
Didymium minus (Lister) Morgan	1	(1)			
Physarum sp. A	1			1	
Physarum rubiginosum Fr. & Palmquist	1		(1)		
Trichia favoginea (Batsch) Pers.	1			1	
Total	244	102 (50)	34 (8)	39 (11)	0 (0)

Table 2 Values of pH recorded for moist chamber cultures prepared with samples of the four different types of leaves collected in 2012 and 2013. Note: DW = dogwood, WO = white oak, RM = red maple and SY = sycamore.

	2012				2013			
	DW	WO	RM	SY	DW	WO	RM	SY
No. of cultures	40	40	40	40	40	40	40	40
Mean pH	6.3	4.6	4.3	6.2	6.6	4.2	5.0	5.4
Maximum pH	6.6	4.8	4.3	6.4	7.1	4.5	5.5	5.8
Minimum pH	6.0	4.4	4.1	5.7	6.3	4.0	4.1	6.3

in the shortest period of time. Sycamore was followed by white oak and then dogwood, with maple requiring the least amount of water to maintain moist conditions in moist chamber cultures prepared with this type of leaf.

Discussion

Several previous studies (e.g., Härkönen 1981, Stephenson 1989, Tran et al. 2008, Rollins & Stephenson 2012) have used the moist chamber technique to document the presence of myxomycetes on dead leaves, and the fact that some species are consistently associated with this type of microhabitat has been noted in monographs (e.g., Martin & Alexopoulos 1969) and ecological treatments (Stephenson 2011) of the group. All of the species represented by an appreciable number of records in the present study typically occur on leaf litter.

Interestingly, one of the species (a stalked *Diderma* with a yellow sporotheca) recorded from dogwood leaves collected in 2012 appears to fit the description of *D. miniatum*. This apparently rare species is known from Mexico, but we are not aware of any previous records from the United States (Hernandez-Cuevas & Estrada-Torres 1997).

The most surprising result obtained relates to the differences in the assemblages associated with different types of leaves and even the same type of leaf in two different years. Percentage similarity and coefficient of community values calculated for the two sets of data (i.e., 2012 and 2013) were 0.25 and 0.50, respectively. As such, the difference between the two years could be attributed more to differences in abundance than to differences in the species present. However, only two species (*Diderma effusum* and *Physarum bivalve*) were recorded from all three types of leaves that yielded sporocarps (i.e., since no sporocarps appeared in moist chamber cultures prepared with leaves from sycamore). Values of percent similarity ranged from 0.17 (dogwood and white oak for samples collected in 2013) to 0.56 (white oak and red maple for samples collected in 2013). The mean value of percent similarity for all pairwise combinations of the three types of leaves for which sporocarps were recorded was 0.32 for the 2013 samples and 0.39 for the 2012 samples.

Species diversity indices calculated for the entire set of data for each of the two years were 1.86 for 2013 and 1.67 for 2012. Values calculated for just one type of leaf ranged from 0.78 (red maple in 2013) to 1.53 (dogwood in 2013). Mean values for the two years (considering only the three types of leaves yielding sporocarps) were 1.24 for 2013 and 1.39 for 2012. As such, species diversity of the assemblages of myxomycetes associated with particular types of dead leaves appears to be uniformly relatively low, presumably because the high level of abundance of one or a very few species in most instances.

As noted earlier, sycamore leaves yielded only plasmodia, and these were restricted to samples collected in 2012. Samples collected in 2013 never displayed any evidence of the presence of myxomycetes. Just why this was the case remains problematic, since in both years all samples were collected from the same study site and handled in the same manner, with the same person (the first author) checking all moist chamber cultures on a regular basis. Sycamore seemed to be characterized by levels of nutrients comparable to those recorded for the other types of leaves, although in a few instances (e.g., nitrogen and manganese) the values obtained for sycamore were lower than those for dogwood, white oak or red maple (Table 3). However, it is possible that physical and not chemical properties of sycamore leaves render them less suitable for the growth and development of myxomycetes. As already noted, sycamore leaves dried out more quickly than the other types of leaves, and this fact might affect the microbes (primarily bacteria) upon which myxomycetes feed. In any case, this is something that warrants additional study.

Table 3 Levels of nutrients in the four types of leaves. The values recorded for samples collected in 2012 are given in parentheses, whereas those outside the parentheses are for samples collected in 2013.Note: DW = dogwood, WO = white oak, RM = red mapleand SY = sycamore.

Type of nutrient	DW	WO	RM	SY
Nitrogen (%)	0.98 (0.55)	1.39 (0.60)	0.72 (1.54)	0.59 (0.46)
Phosphorus (%)	0.08 (0.05)	0.11 (0.04)	0.07 (0.09)	0.06 (0.05)
Magnesium (%)	0.15 (0.17)	0.15 (0.13)	0.36 (0.11)	0.21 (0.14)
Potassium (%)	0.2 (0.09)	0.67 (0.09)	0.59 (0.09)	0.78 (0.07)
Calcium (%)	1.97 (2.72)	0.81 (1.37)	1.49 (0.88)	1.55 (1.95)
Sulfur (%)	0.09 (0.07)	0.09 (0.06)	0.08 (0.11)	0.13 (0.1)
Boron (ppm)	17.4 (33.6)	16.3 (24.7)	28.6 (13.3)	20.1 (34.5)
Iron (ppm)	5070 (147)	386 (139)	311 (243)	173 (476)
Manganese (ppm)	344 (262)	1740 (2369)	1620 (632)	293 (252)
Copper (ppm)	20.1 (26.8)	12.5 (31.6)	15.5 (22.6)	10.9 (52.7)
Zinc (ppm)	26.5 (27.0)	18.2 (23.2)	42.9 (28.4)	10.9 (28.4)
Aluminum (ppm)	4200 (652)	359 (190)	765 (259)	109 (466)

The most important results of the present study were that (1) different types of leaves were found to be characterized by different assemblages of myxomycetes and (2) the assemblages present are not necessarily the same from year to year. The latter is something that should be considered when carrying out surveys of myxomycetes at a particular locality. Numerous published surveys have involved collecting samples for subsequent preparation of moist chamber cultures on a single occasion, which would capture only a subset of the total assemblage of species present. Although this fact is generally acknowledged, the data presented herein would appear to indicate that it may represent more of a factor for the litter microhabitat than usually recognized.

References

Gauch HG Jr 1982 – Multivariate analysis in community ecology. Cambridge University Press, Cambridge.

Gilbert HC, Martin GW 1933 – Myxomycetes found on the bark of living trees. University of Iowa Studies in Natural History 15, 3–8.

Härkönen M 1981 – Myxomycetes developed on litter of common Finnish trees in moist chamber cultures. Nordic Journal of Botany 1, 791–794.

Hernandez-Cuevas L, Estrada-Torres A 1997 – Mexican Didymiaceae (Myxomycetes, order Physarales): three new records and comments on *Mucilago crustacea*. Mycotaxon 62, 319–335.

Lado C 2005-2013 – An online nomenclatural information system of Eumycetozoa. http://www.nomen.eumycetozoa.com (accessed on Jan. 15, 2013).

Martin GW, Alexopoulos CJ 1969 – The myxomycetes. University of Iowa Press, Iowa City.

Mueller-Dombois D, Ellenberg H 1974 – Aims and methods of vegetation ecology. John Wiley and Sons, New York.

Rollins AW, Stephenson SL – 2012. Myxogastrid distribution within the leaf litter microhabitat. Mycosphere 3, 543–549.

Shannon CE, Weaver W 1963 – The mathematical theory of communication. University of Illinois Press, Irbana, Illinois.

Stephenson SL1988 – Distribution and ecology of myxomycetes in temperate forests. I. Patterns of occurrence in the upland forests of southwestern Virginia. Canadian Journal of Botany 66, 2187–2207.

Stephenson SL 1989 – Distribution and ecology of myxomycetes in temperate forests. II. Patterns of occurrence on bark surface of living trees, leaf litter, and dung. Mycologia 81, 608–621.

Stephenson SL 2011 – From morphological to molecular: studies of myxomycetes since the publication of the Martin and Alexopoulos (1969) monograph. Fungal Diversity 50, 21–34.

Stephenson SL, Stempen H 1994 –Myxomycetes: a handbook of slime molds. Timber Press, Portland, Oregon.

Tran HTM, Stephenson SL, Hyde KD, Mongkolporn O 2008 – Distribution and occurrence of myxomycetes on agricultural ground litter and forest floor litter in Thailand. Mycologia 100, 181–190.