Myxomycetes associated with woody twigs

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REVISTA MEXICANA DE MICOLOGÍA 27: 21-28, 2008

ORIGINAL

Myxomycetes asociados con ramitas leñosas

Resumen. La técnica de la cámara húmeda fue utilizada con el objetivo de investigar las especies de mixomicetes (hongos mucilaginosos plasmodiales o mixogástridos) asociadas con el microhábitat representado por pequeñas ramas leñosas caídas en el suelo. Muestras de ramitas (<1.0 cm de diámetro) fueron recolectadas en Kansas, Oklahoma, Arkansas y Virginia en los Estados Unidos, Costa Rica, Nueva Zelanda, Australia y el sur de Argentina. La mayoría (67%) de las 256 cámaras húmedas preparadas mostraron alguna evidencia (ya sea cuerpos fructíferos o plasmodios) de mixomicetes. En general, las ramitas de los bosques caducifolios de zonas templadas fueron más productivas que las recolectadas en otros tipos de bosque. El grupo menos productivo de muestras (23% de muestras positivas a partir de 47 cámaras húmedas y solo cinco especies) fue recolectado en un bosque de encinos de zonas altas (3120 m) en Costa Rica. Por el contrario, dos grupos de cámaras húmedas preparados con muestras obtenidas en bosques caducifolios de zonas templadas dieron como resultado >85% de cámaras positivas y >15 especies. Entre las especies de mixomicetes registradas en ramitas se incluye *Arcyria cinerea* (de la que se obtuvieron el mayor número de colecciones), *Stemonitis fusca* var. *nigrescens*, *Perichaena depressa*, *Perichaena chrysosperma* y *Physarum pusillum*.

Palabras clave: ecología, mixomicetes, hongos mucilaginosos, ramitas.

Abstract. The moist chamber culture technique was used to investigate the assemblage of myxomycetes (plasmodial slime molds or myxogastrids) associated with the microhabitat represented by fallen woody twigs. Samples of twigs (<1.0 cm in diameter) were collected from study areas in Kansas, Oklahoma, Arkansas and Virginia in the United States, Costa Rica, New Zealand, Australia and southern Argentina. A majority (67%) of the 256 cultures prepared with twigs yielded some evidence (either fruiting bodies or plasmodia) of myxomycetes. As a general observation, twigs from temperate deciduous forests were more productive than twigs collected in other types of forests or woodlands. The least productive set of samples (23% positive for 47 cultures and just five species) was collected from a high-elevation (3120 m) oak forest in Costa Rica. In contrast, two sets of cultures prepared with samples obtained from temperate deciduous forests yielded >85% positive cultures and >15 species. The species of myxomycetes recorded from twigs included *Arcyria cinerea* (represented by the largest number of collections), *Stemonitis fusca* var. *nigrescens*, *Perichaena depressa*, *Perichaena chrysosperma* and *Physarum pusillum*.

Key words: ecology, myxomycetes, slime molds, twigs.

Recibido 23 de febrero 2006; aceptado 25 de enero 2008.
Received 23 February 2006; accepted January 25 2008.

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Introduction

Myxomycetes (plasmodial slime molds or myxogastrids) are common inhabitants of forest ecosystems throughout the world [12]. Most ecological studies of these organisms have focused on those species characteristically associated with decaying wood or bark. The myxomycetes found in such microhabitats often occur in great profusion, typically producing fruiting bodies of sufficient size to be easily detected in the field [7, 8]. However, several other microhabitats for myxomycetes are found in forest ecosystems. The two most important of these are the bark surface of living trees and the layer of leaf litter on the forest floor. In tropical forests, aerial litter (dead but still attached plant parts above the ground) represents another important microhabitat for myxomycetes [10].

The association of some myxomycetes with litter is well known [2], but only a few studies [3, 9, 11] have examined the assemblages of species found in this microhabitat. Results from the most extensive of these studies [9] suggest that for at least the more common species, the myxomycetes occurring on litter are not the same species found in other microhabitats in the same forest community. The litter layer on the forest floor consists mostly of dead leaves, but it also contains other types of plant debris (e.g., pieces of bark, fragments of wood, fruits, seeds, inflorescences, and small woody twigs). On numerous occasions for series of moist chamber cultures prepared with samples of litter, the senior author has observed myxomycetes fruiting on small fragments of bark or pieces of twigs within a particular culture. In almost every instance, the fruiting was small (usually no more than one or a few fruiting bodies) and restricted to the fragment of bark or piece of twig. Since myxomycetes are known to exhibit differential patterns of distribution with respect to the various types of microhabitats available to them [9, 10], an obvious question is whether or not a distinct assemblage of species tends to be associated with either of these components of the litter layer. Of the two, twigs are much more common. In forest communities, small twigs (defined herein as <1.0 cm in diameter) are often abundant on the forest floor (Figure 1). The objective of the present study was to characterize the assemblage of myxomycetes associated with the twig microhabitat in a number of different types of forests and woodlands throughout the world. Although fruiting bodies of myxomycetes that develop under natural conditions sometimes occur on twigs, no effort was made to selectively examine this microhabitat in the field. Instead, the moist chamber culture technique as it applies to the study of myxomycetes [12] was used to generate the data reported in this paper.

Materials and methods

Study areas

Samples of twigs were collected during the period of 2003 to 2005 from study areas in the United States of America, Costa Rica, New Zealand, Australia and southern Argentina (Table 1). These study areas are described below. The abbreviation used to denote a particular study area in Tables 1 and 2 is indicated in each instance. Communities characterized by woody plants of sufficient size and abundance to produce a closed canopy condition were classified as forests, whereas those communities with smaller, more scattered woody plants were considered to be woodlands.

Figure 1. Twigs on the forest floor of the study area in southern New Zealand.

Table 1. Summary data on moist chamber cultures prepared with woody twigs

Study area	Number of cultures	Positive (%)	pH mean	pH range	Number of species	Species per culture
Arkansas, USA (AR-1)	16	63	4.7	4.1 to 5.1	4	0.9
Arkansas, USA (AR-2)	11	100	5.7	3.7 to 6.5	8	1.6
Australia (AU-1)	25	68	6.4	4.4 to 7.5	6	0.8
Australia (AU-2)	24	67	5.9	4.5 to 6.6	11	1.0
Australia (AU-3)	14	86	5.4	5.1 to 5.5	6	1.6
Costa Rica (CR-1)	23	43	7.8	7.1 to 8.1	4	0.5
Costa Rica (CR-2)	47	23	6.2	4.3 to 6.8	5	0.2
Kansas, USA (KP)	31	87	5.9	5.1 to 6.4	16	1.6
Oklahoma, USA (KS)	11	73	4.7	5.0 to 6.0	8	1.5
New Zealand (NZ)	16	88	5.1	4.6 to 5.3	2	1.0
Argentina (SA)	5	100	5.1	5.0 to 5.2	4	1.8
Virginia, USA (VA)	33	91	5.1	4.3 to 5.7	20	2.3
Total/mean	256	67	5.7	3.7 to 8.1	43	1.1

ARGENTINA

Tierra del Fuego National Park in southern Argentina (54° 50' S, 68° 34 E), elevation 50 m, southern beech forest [SA].

AUSTRALIA

Roadside community along the Ross Highway east of the city of Alice Springs in central Australia (23° 45' S, 133° 55' E), elevation 600 m, acacia woodland-grassland [AU-1].

Roadside community along the Stuart Highway north of the city of Alice Springs in central Australia (23° 26' S, 133° 49 E), elevation 600 m, acacia woodland-grassland [AU-2].

Waste Point near the city of Jindabyne in the Snowy Mountains of southeastern Australia (36° 21' S, 148° 36 E), elevation 990 m, eucalyptus woodland [AU-3].

COSTA RICA

Reserva Leonel Oviedo of the University of Costa Rica Campus at San Pedro de Montes de Oca, Costa Rica (9° 56' N, 84° 03 W), elevation 1305 m, secondary tropical forest [CR-1].

Cerro de la Muerte Biological Station on the eastern slope of Cerro Bellavista, Perez Zeledón, Costa Rica (9° 33' N, 83° 44 W), elevation 3120 m, oak forest [CR-2].

NEW ZEALAND

Kepler Track near Lake Te Anou in southwestern New Zealand (45° 26' S, 167° 41 E), elevation 215, southern beech forest [NZ].

UNITED STATES OF AMERICA

Devil's Den State Park in northwestern Arkansas, USA (35° 47' N, 94° 15 W), elevation 506 m, oak-hickory forest [AR-1].

Roadside near Birdtown in north central Arkansas, USA (35° 18' N, 92° 36 W), elevation 150 m, pine forest [AR-2].

Konza Prairie in eastern Kansas, USA (39° 06' N, 96° 36 W), elevation 310 m, gallery forest dominated by black walnut and hackberry [KP].

Keystone Natural Area in northeastern Oklahoma, USA (36° 11' N, 96° 14 W), elevation 335 m, old-growth oak-hickory forest [KS].

Farm woodlot located south of the city of Bedford in south central Virginia, USA (37° 43' N, 79° 34 W), elevation 275 m, mixed hardwood forest [VA].

Moist chamber cultures

All twigs used to prepare moist chamber cultures were <1.0 cm in diameter. Except for a single study area (AR-2) in Arkansas, USA, where the samples collected included some twigs from *Pinus* (pine), all twigs were those of broadleaf trees. The vast majority of twigs still had a covering of bark present; only a few examples were decorticated. In each study area, twigs of the prerequisite size were selected randomly, placed in small paper bags and transported to the laboratory. Moist chamber cultures were prepared in the manner described by Stephenson and Stempen [12]. Twigs were broken as necessary to produce enough pieces of the right length to cover the bottom of a particular moist chamber. The moist chambers used consisted of disposable plastic Petri dishes (10 cm diam) lined with filter paper. Twigs were moistened with distilled water. After a period of approximately 24 hours, the pH of each culture was measured using a flat surface electrode and a Fisher Accument Model 610A pH meter. After pH had been determined, excess water in each dish was poured off. Cultures were kept at room temperature (22-25 C) in diffuse daylight and examined with a dissecting microscope every week for a period of approximately six weeks in order to detect plasmodia and/or fruiting bodies of myxomycetes. After the cultures dried out, they were rewetted and examined at less frequent intervals for another two to three months.

Myxomycete plasmodia and/or fruiting bodies were noted and recorded each time a culture was checked. When fruiting bodies of a given species developed more than once in the same culture, they were considered to represent a single record. As soon as the fruiting bodies were judged to be fully mature, the twig or (more often) portion of the twig upon which the fruiting occurred was removed from the moist chamber culture, allowed to dry and then glued in a small paper box suitable for long-term storage. Identifications of collections were made using the descriptions and keys provided in Martin and Alexopoulos [7] and other more recent monographs. Vouchers were deposited in the mycological herbarium of the University of Arkansas (UARKM). Nomenclature used herein follows Lado [5] and Hernández-Crespo and Lado [4], with the conserved names of several genera [6] approved recently by the Committee for Fungi [1] of the IAPT.

Results

Samples of twigs collected in the various study areas were used to prepare a total of 256 moist chamber cultures. The majority of these (67%) yielded some evidence (either fruiting bodies or plasmodia) of myxomycetes (Table 1), with the actual percentage of positive cultures for a particular study area ranging from 23% to 100%. The values of pH recorded for cultures prepared with twigs varied widely (range = 3.7 to 8.1, with an overall mean of 5.7). A set of cultures prepared with twigs from a tropical forest in Costa Rica had the highest values (mean pH = 7.8), whereas sets of cultures prepared with twigs from Oklahoma, USA and one of the two study areas in Arkansas, USA were the most acidic (mean pH = 4.7).

The number of cultures prepared for a particular study area ranged from five to 47. Larger sets of cultures might be expected to yield more species, but the correlation coefficient value ($r^2 = 0.15$, $P>0.05$) calculated for these data indicated that the number of species recovered was not significantly correlated with the number of cultures. However, the low species totals recorded for sets of samples from New Zealand (16 cultures and only two species) and the second of the two study areas in Costa Rica (47 cultures and only five species) would seem to reflect actual low diversity of the myxomycetes present.

The number of species recorded per culture (Table 1) varied from a low of 0.2 for the same study area in Costa Rica (noted above) that produced a total of only five species to a high of 2.3 for the study area in Virginia, USA. As a general pattern, temperate deciduous forests (mean of 1.6 for five study areas) were characterized by values higher than those recorded for other vegetation types (mean of 1.0 for seven study sites). The very low values (0.5 and 0.2) recorded for the two study areas in Costa Rica would seem noteworthy. The first study area was a secondary tropical forest, whereas the second was located at the highest elevation (3120 m) sampled in the present study.

At least 43 species of myxomycetes in 17 genera were recorded from the 256 moist chamber cultures (Table 2). Several of the species recovered were represented by exceedingly limited material and the identification given in Table 2 is tentative. In five instances, only identification to genus was possible. However, it could be determined that the species involved was clearly different from any of the other already identified members of the same genus. The 43 species included representatives of five orders of myxomycetes, with only members of the order Ceratiomyxales not recorded. The Physarales, Trichiales and Stemonitales were the predominant orders, with each contributing >20% of the total number of species. The Physarales alone constituted almost 40% of all species recorded, and 10 of these were members of the genus *Physarum*. *Arcyria cinerea* was the species represented by the largest number of collections (50) and was recorded from eight of the 12 study areas; *Stemonitis fusca* var. *nigrescens* was represented by fewer collections but was recorded from 10 study areas. Only three other species (*Perichaena depressa*, *Perichaena chrysosperma* and *Physarum pusillum*) were recorded from as many as five different study areas. Just over half (51%) of the 43 species appearing on the samples of twigs collected in the present study were recovered from only a single study area.

Discussion

As a general observation, twigs from temperate deciduous forests were more productive than twigs collected in other types of forests or woodlands. The least productive set of samples (23% positive cultures, just five species and only 0.5 species per culture) was collected from a high-elevation oak forest (CR-2) in Costa Rica, but a second set of samples from a secondary tropical forest in Costa Rica (CR-1) had only 43% positive cultures, four species and only 0.2 species per culture. In contrast, two sets of samples from temperate deciduous forests (KP and VA) yielded >85% positive cultures and the highest species totals (16 and 20) recorded for any of the study areas. However, a set of samples from a deciduous forest in Arkansas, USA (AR-1) was relatively less productive (67% positive cultures and just four species). The high species totals for the first two sets of samples would seem to reflect, at least in part, the high overall diversity of the woody plants contributing twigs in these two study areas (Stephenson, personal observation). This apparent pattern of increasing diversity of myxomycetes with increasing diversity of woody plants is probably not surprising. Stephenson [9] reported the same type of pattern for the assemblages of myxomycetes associated with forest floor litter in five study areas in southwestern Virginia, USA. Additional evidence is provided examination of the species totals recorded from study areas in which the total number of species of woody plants present was very low (essentially only a single species), which was the case for the study area (NZ) in southern New Zealand.

The assemblage of myxomycetes recorded from twigs contains only 16 of the 34 species reported by Stephenson [9] for the litter microhabitat of the five study

Table 2. Occurrence of myxomycetes in moist chamber cultures prepared with twigs collected in the various study areas

Species	Study area												Total no. of study areas
	AR-1	AR-2	AU-1	AU-2	AU-3	CR-1	CR-2	KP	KS	NZ	SA	VA	
Arcyria cinérea	P	P				P	P	P	P		P	P	8
A. denudata	P											P	2
A. insignis										P		P	2
A. pomiformis		P											1
Arcyria sp. A							P						1
Arcyria sp. B												P	1
Clastoderma debaryanum		P										P	2
Collaria arcyrionema				P								P	2
Comaticha tenerrima												P	1
C. nigra		P		P							P		3
C. pulchella			P	P					P			P	4
Comatricha sp. A												P	1
C. laxa			P	P	P				P				4
Cribraria violacea								P					1
Diderma chondrioderma								P					1
D. effusum											P	P	2
Didymium cf. *anellus*				P					P				2
D. minus				P									1
D. squamulosum						P							1
Echinostelium corynophorum												P	1
E. minutum		P											1
Fuligo séptica								P					1
Licea biforis		P											1
Metatrichia vesparia												P	1
Oligonema schweinitzii					P								1
Perichaena chrysosperma					P	P		P	P			P	5
P. depressa			P		P	P	P	P					5
P. vermicularis				P									1
Physarum album								P					1
P. bivalve								P					1
P. cinereum			P		P			P					3
P. compressum							P						1
P. galbeum												P	1
P. melleum		P						P	P				3
P. pusillum		P	P	P				P				P	5
P. serpula				P				P					2
P. viride								P				P	2
Physarum sp. A				P									1
Stemonitis flavogenita									P			P	2
S. fusca var. *nigrescens*	P		P	P	P		P	P	P	P	P	P	10
Stemonitis sp. A												P	1
Trichia favoginea	P							P					2
Willkommlangea reticulata								P				P	2
Total	4	8	6	11	6	4	5	16	8	2	4	20	

Note: P = present

areas in southwestern Virginia, USA mentioned above. Interestingly, *Arcyria cinerea*, the species represented by the most collections in the present study, also was the single most important species appearing on litter and occurred in 43% of all cultures. However, the other more important species recorded from twigs were either not among those reported in the earlier study or were much less common. For example, *Stemonitis fusca* var. *nigrescens* was common on twigs but was not recorded from samples of litter. The same was true for *Perichaena depressa* and *Physarum pusillum*. A number of other species (e.g., *Clastoderma debaryanum*, *Comatricha laxa*, *Diderma effusum*, *Echinostelium minutum*, *Perichaena chrysosperma* and *Physarum viride*) were recorded from both litter and twigs but only one of these (*D. effusum*) was common on either substrate. *Diderma effusum* was one of the more characteristic species associated with litter in the earlier study but was recorded only twice from twigs. Overall, cultures prepared with litter tended to be more productive (>75% of all cultures) than those prepared with twigs in the present study (67%), but if only those study areas in temperate deciduous forests are considered, then twigs are more productive than litter.

Several of the species recorded from twigs (Table 2) are lignicolous forms usually associated with coarse woody debris [7]. Their occurrence on woody twigs is probably not surprising, but the fact that they appeared in moist chambers would seem noteworthy, based on observations by the senior author over a period of more than 30 years. Prominent examples of these species include *Fuligo septica*, *Metatrichia vesparia* and *Trichia favoginea*. Special mention should be made of *Willkommlangea reticulata* (Figure 2). This species is listed from only two study areas in Table 2. However, it also has been recorded (Stephenson, unpub. data) in moist chamber culture on samples of twigs collected from two additional study areas in northwest Arkansas, USA that were not part of the project reported herein. Moreover, during the course of recent field collecting carried out in southern

Figure 2. Fruiting bodies of *Willkommlangea reticulata*, a myxomycete for which woody twigs may represent the primary microhabitat.

Argentina, a series of specimens of this relatively rare species was collected, and virtually all of these were associated with twigs. Although our data are limited, there is at least some evidence to suggest that twigs may represent the primary microhabitat for *W. reticulata*.

In conclusion, results of the present study suggest that the myxomycetes associated with the microhabitat represented by woody twigs are not necessary the same as those associated with ground litter as a whole. As such, these data provide additional evidence that these organisms are not generalists and, instead, exhibit differential patterns of distribution with respect to the various types of microhabitats potentially available to them in terrestrial ecosystems.

Acknowledgments

The study reported herein was carried out in the context of other larger projects supported in part by grants from the National Science Foundation (especially DEB-0316284) and the Australian Biological Resources Study Participatory Program. Appreciation is extended to John Shadwick for supplying the image used for Figure 2.

References

1. Gams, W., 2005. Report of the Committee for Fungi: 13. Taxon 54: 828-830.
2. Gray, W.D., C.J. Alexopoulos, 1968. Biology of the Myxomycetes. Ronald Press, New York.
3. Härkönen, M., 1981. Myxomycetes developed on litter of common Finnish trees in moist chamber cultures. Nordic Journal of Botany 1: 791-794.
4. Hernández-Crespo, J. C., C. Lado, 2005. An on-line nomenclatural information system of Eumycetozoa. http://www.nomen.eumycetozoa.com (24-X-2005).
5. Lado, C., 2001. Nomenmyx. A Nomenclatural Taxabase of Myxomycetes. Cuadernos de Trabajo Flora Micológica Ibérica 16:1-221.
6. Lado, C., U. Eliasson, S.L. Stephenson, A. Estrada-Torres, M. Schnittler, 2005. (1688-1691) Proposals to conserve the names *Amaurochaete* against *Lachnobolus*, *Ceratiomyxa* against *Famintzinia*, *Cribraria* Pers. against *Cribraria* Schrad. ex J. F. Gmel. And *Hemitrichia* against *Hyporhamma* (Myxomycetes). Taxon 54: 543-545.
7. Martin, G.W., C.J. Alexopoulos, 1969. The Myxomycetes. University of Iowa Press, Iowa.
8. Stephenson, S.L., 1988. 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.
9. Stephenson, S.L., 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.
10. Stephenson, S.L., A. Estrada-Torres, M. Schnittler, C. Lado, D. Wrigley de Basanta, N. Ogata, 2003. Distribution and ecology of myxomycetes in the forests of Yucatan. *In*: Gómez-Pompa A., M. Allen, S. Fedick, J. Jimenez (eds.), Lowland Maya Area: Three Millennia at the Human-Wildland Interface. Haworth Press, New York. Pp. 241-259
11. Stephenson, S.L., J.C. Landolt, D.L. Moore, 1998. Protostelids, dictyostelids, and myxomycetes in the litter microhabitat of the Luquillo Experimental Forest, Puerto Rico. Mycological Research 103: 209-214.
12. Stephenson, S.L., H. Stempen, 1994. Myxomycetes: A Handbook of Slime Molds. Timber Press, Portland, Oregon.