ISSN 2215-650X
Slime Molds
Slime Molds **3** (2023) V3A12 https://doi.org/10.5281/zenodo.7725786

Myxomycetes associated with *Nowellia curvifolia* on decorticated spruce logs

Steven L. Stephenson[1] and Randy G. Darrah[2]

[1] Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas 72701
[2] P.O. Box 391, Farmington, West Virginia 26571

E-mail: slsteph@uark.edu

Received: 16 February 2023
Accepted for publication: 11 March 2023
Published: 24 March 2023
Editor: Nikki H.A. Dagamac

Abstract: The evergreen leafy liverwort *Nowellia curvifolia* often forms a nearly complete cover over the surface of decorticated red spruce (*Picea rubens*) logs in the high-elevation forests of the Central and Southern Appalachians. Samples of dead wood with *Nowellia* present were collected from an old-growth red spruce forest in West Virginia and used to prepare a series of 50 moist chamber cultures. Forty-seven (94%) of these cultures produced evidence (either plasmodia or fruiting bodies) of myxomycetes. A total of ten species in nine genera was recorded, with *Cribraria microcarpa* (68% of all cultures) the overwhelming dominant. The ecological aspects of the substrate complex involving *Nowellia* on decorticated spruce logs is discussed.

Keywords: ecology, moist chamber cultures, North America, old-growth forest

Introduction

The evergreen leafy liverwort *Nowellia curvifolia* (Dicks.) Mill. (phylum Hepatophyta; order Jungermanniales) often forms a nearly complete cover over the surface of decorticated red spruce (*Picea rubens* Sag.) logs in the high-elevation forests of the Central and Southern Appalachians (Fig. 1). Schnittler et al. (2000) reported that the myxomycete *Barbeyella minutissima* Mel. is consistently associated with the microhabitat represented by leafy liverworts on decorticated logs in montane coniferous forests in temperate regions of the Northern Hemisphere. In eastern North America, the leafy liverwort involved is almost invariably *Nowellia curvifolia*. They also indicated that several other species of myxomycetes (i.e., *Colloderma oculate* [C. Lippert] G. Lister, *Lamproderma columbinum* [Pers.] Restaff., and *Epiderma tigrinum* [Schrad.] Rostam.) tend to co-occur with *B. minutissima*.

Because of the consistent association of these species with bryophytes, they are regarded as bryophilous ("bryophyte loving") as noted by Ing (1994). However, the relationship between the two organisms may involve more than just the presence of a bryophyte. Schnittler et al. (2000) noted that there is often a thin, shiny layer over the surface of the wood on which *Nowellia curvifolia* and *Barbeyella minutissima* occur. They suggested that this layer appeared to consist of various algae and bacteria, and it was possible that these might represent a food resource for myxomycetes. Smith and Stephenson (2007) examined this slimy layer and were able to isolate and identify the major taxa present. They found that the slimy layer was dominated by just three taxa—two cyanobacteria (*Thermococcus tenax* [Kirchner]

Hieronymus) and *Panthera saxicola* Naeg.) and one green alga (*Clorococcum humicola* [Naeg.] Rabenhor). The relative abundance of these organisms suggested that they could represent a possible food source for certain species of myxomycetes.

Figure 1. *Nowellia curvifolia* on a decorticated spruce log.

The purpose of the present study was to use the moist chamber technique to characterize the assemblage of myxomycetes associated with the substrate complex consisting of (1) decaying spruce logs in which the bark has been lost, (2) the often nearly complete cover of the leafy liverwort *Nowellia curvifolia* over the surface of the log, and (3) an assemblage of cyanobacteria, bacteria, and green algae. There are only two reports of *Barbeyella minutissima* appearing in moist chamber culture (Stephenson et al. 2019), and the other species of myxomycetes mentioned above are among those either not known to produce fruiting bodies in moist chamber cultures or do so only on very rare occasions. As such, records of these species are based almost solely on specimens collected from the field. However, the fact that such myxomycetes are associated with the substrate complex described above suggests that the latter supports additional species that are more likely to be recorded in moist chamber cultures than as specimens that develop under field conditions. To our knowledge this substrate complex has never been investigated with the moist chamber culture technique, which prompted the study reported herein.

Methods and Materials

The samples used in the present study were collected on 31 August 2022 from the old-growth red spruce forest in the Gaudineer Scenic Area (38°37′4″ N, 79°50′33″ W; elevation 1220 m) on Cheat Mountain in Randolph and Pocahontas Counties in West Virginia. This study site was described in detail by Adams and Stephenson (1989). All samples consisted of small pieces of wood taken from the surface of decorticated spruce logs with a nearly complete cover of *Nowellia curvifolia* present. All the logs from which samples were collected were relatively large, with most more than 40 cm in diameter. All samples were sent to the Eumycetozoan Laboratory at the University of Arkansas, where they were used to prepare

fifty moist chamber cultures in the manner described by Stephenson and Stempen (1994). Each moist chamber consisted of a disposable plastic Petri dish (90 mm diameter) lined with filter paper. Enough sample material (typically three to five pieces) was placed in each dish to cover the bottom, and then this material was moistened with distilled water. After a period of approximately 24 hours, the pH of a small subset (n = 10) of the cultures was determined with a portable pH meter, and excess water in all the Petri dishes was poured off. Moist chamber cultures were placed out of direct sunlight, maintained at room temperature, and checked for evidence of myxomycetes (either plasmodia or fruiting bodies) each week for a period of more than five months. When the fruiting bodies of myxomycetes were detected, they were removed, allowed to dry at room temperatures and placed in small pasteboard boxes for permanent storage.

Prior to being used to prepare the moist chamber cultures, all samples of wood were carefully examined under a microscope to determine if the fruiting bodies of any myxomycetes were already present. These were collected and placed in small pasteboard boxes in the same manner as specimens appearing later in the moist chamber cultures.

Results

Forty-seven (94%) of the moist chamber cultures prepared in the present study were positive for myxomycetes, and 39 (78%) of the cultures produced fruiting bodies. Eight cultures (16%) produced only plasmodia that did not fruit. Ten species in nine genera were represented among the 54 specimens collected from the moist chamber cultures. *Cribraria macrocarpa* was by far the most abundant species and appeared in 34 (68%) of all cultures. Five other species were recorded from at least two cultures.

List of species

All species of myxomycetes recorded in the moist chamber cultures prepared in the present study are listed by genus and species in the list provided below. One or more voucher specimens are given for each species and comments are provided on species of particular interest. The nomenclature used follows Lado (2005-2023). Collection numbers are those of the first co-author.

Arcyria cinerea (Bull.) Pers. (six specimens, including SLS 22-09 and SLS 22-20). This species is one of the most common of all myxomycetes and has been recorded from virtually all surveys ever carried out.

Arcyria denudata (L.) Wettst. (one specimen, SLS 22-19)

Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr. (one specimen, SLS 22-17) Although traditionally considered to be a myxomycete, molecular data indicate that members of the genus *Ceratiomyxa* belong to a different group of eumycetozoans. However, *C. fruticulosa* is usually included in surveys carried out for myxomycetes, which is the approach used in the present study. The single specimen appeared more than five months after the moist chambers cultures had been prepared.

Colloderma oculatum (C. Lippert) G. Lister (three specimens, including SLS 22-16)

Comatricha nigra (Pers. Ex J.F. Gmel.) J. Schröt. (two specimens, including SLS 22-12)

Cribraria microcarpa (Schrad.) Pers. (34 specimens, including SLS 22-06 and SLS 22-18)

Diderma effusum (Schwein.) Morgan (two specimens, including SLS 22-13)

Hemitrichia decipiens (Pers.) Garcia-Cunch, J.C. Zemera & Lado (three specimens, including SLS 22-15). This species is listed as *Trichia decipiens* Pers. in all but the most recent treatments of the myxomycetes.

Licea minima Fr. (one specimen, SLS 22-17)

Physarum viride (Bull.) Pers. (one specimen, SLS 22-01)

Discussion

The high percentage (94%) of positive cultures in the present study clearly indicates that the substrate complex represented by *Nowellia curvifolia* occurring over the surface of decorticated spruce logs is favorable for myxomycetes. However, the assemblage present is not especially diverse, with only ten species recorded in the present study. It is possible that the relatively low pH (4.9 ± 0.1 for the samples collected in the present study) of the decayed wood upon which *Nowellia* is typically found would restrict the occurrence of some species of myxomycetes. It has long been known that pH is a major factor affecting the distribution of these organisms in nature (Stephenson 1988, Stephenson and Stempen 1994).

The moist chamber cultures were prepared on 7 October 2022, and the first small plasmodia appeared after one week. After two weeks, plasmodia were present in 20% of all cultures. Many of the plasmodia that developed in the moist chamber cultures were still active but had not produced fruiting bodies after five months. More than just a few of the plasmodia reached an appreciable size (often >1.0 cm in total extent) and based on their color almost certainly represented several different species. This suggests that not all species of myxomycetes associated with the substrate complex involving *Nowellia curvifolia* were recorded.

As noted earlier, prior to being used to prepare the moist chamber cultures, all samples were carefully examined under a microscope to determine if the fruiting bodies of any myxomycetes were already present. This examination revealed the occurrence of three old fruiting bodies of *Hemitrichia decipiens* and a small cluster of stipes of what presumably species of *Stemonitis* tentatively identified as *S. flavogenita*. Interestingly, the former was one of the species recorded from the moist chamber cultures.

The abundance of *Cribraria microcarpa* in the present study was unexpected. This species is cosmopolitan, but the fruiting bodies "are so inconspicuous as to be overlooked easily in the field" (Martin and Alexopoulos (1969). The results of the present study at least suggest that it is much more abundant than published records from studies involving only field collected specimens would indicate. Obviously, this is almost surely the case for other species of myxomycetes that produce very small fruiting bodies.

During late summer and early fall, examination of decaying spruce logs will almost invariably yield numerous fruiting of myxomycetes. It is possible that the presence or absence of *Nowellia* could be factor. Stephenson and Rojas (2019) reported that mosses are effective traps for the spores of myxomycetes. They suspended nylon mesh bags containing autoclaved samples of mosses from low-hanging branches in in the understory of a forest. These were left in place for several months. When the samples of mosses were removed from the mesh bags and used to prepare moist chamber cultures, 95 percent of these cultures were positive for myxomycetes. Although *Nowellia* potentially could serve as an effective spore trap, the myxomycetes associated with the microhabitat complex of for which this liverwort is one component may have been derived from another source. Feest et al. (2015) demonstrated that the amoeboflagellates of myxomycetes are consistently present (47% of the wood samples they examined), in dead wood. As such, they would be expected to occur in the decorticated wood that represents the underlying substrate of *Nowellia*. Interesting, there is one other feature that might make *Nowellia* an unusually favorable microhabitat for myxomycetes. The base of each of the bi-lobed leaves of *Nowellia*

is folded over to form what has been referred to as a "water-sac" (Schertler 1977). This miniature pouch-like structure retains moisture even when the remining surface of the leaf dries out. Although this has never been demonstrated, it would seem possible that the water-sac could represent a microhabitat in which the amoeboflagellates of myxomycetes could survive. Protozoans and rotifers have been reported to co-occur with other leafy liverworts (J. M. Glime, per. communication), and the association of various microorganisms with mosses is well-established. This is one aspect of what might be termed the "microecology" of myxomycetes that warrants additional study.

Acknowledgments

The research described herein was supported in part by the Slime Mold Project at the University of Arkansas. Appreciation is extended to Dr. Janice M. Glime for information relating to the co-occurrence of protozoans and rotifers with leafy liverworts.

References

Adams HS, Stephenson SL. 1989. Old-growth red spruce communities in the mid-Appalachians. Vegetatio 85: 45-56.

Feest A, Taylor KM, Stephenson SL. 2015. The occurrence of myxomycetes in wood? Fungal Ecol. 17: 179-182.

Ing B. 1994. The phytosociology of myxomycetes. New Phytol. 126: 175-201.

Lado C [Internet]. 2005-2023. An on-line nomenclatural information system of Eumycetozoa, Madrid: Real Jardin Botánico de Madrid, CSIC; [visited 12 Feb 2023]. Available from: http://www.eumycetozoa.com

Martin GW, Alexopoulos CJ, Farr ML. 1983. The Genera of Myxomycetes. University of Iowa Press, Iowa City.

Schertler MM. 1977. Morphology and developmental anatomy in the leafy hepatic *Nowellia curvifolia* (Dick.) Mitt. J Hattori Bot Lab. 42: 241-271.

Schnittler M, Stephenson SL, Novozhilov YK. 2000. Ecology and world distribution of *Barbeyella minutissima* (Myxomycetes). Mycol Res. 104: 1518-1523.

Smith T, Stephenson SL. 2007. Algae associated with myxomycetes and leafy liverworts on decaying spruce logs. Castanea 72: 50-57.

Stephenson SL. 1988. Distribution and ecology of myxomycetes in temperate forests. I. Patterns of occurrence in the upland forests of southwestern Virginia. Canad J Bot. 66: 2187-2207.

Stephenson SL, Stempen H. 1994. Myxomycetes: A Handbook of Slime Molds. Portland: Timber Press. 200 pp.

Stephenson SL, Novozhilov YK, Almadrones-Reyes KJ, Dagamac NHA, Schnittler M. 2019. New records of *Barbeyella minutissima* (Myxomycetes, Echinosteliales) with an updated distribution map. Nova Hedwigia 109: 177-186.

Stephenson SL, Rojas C. 2020. Mosses as spore traps for myxomycetes. Sydowia 72: 215-219.