ISSN 2215-650X
Slime Molds
Slime Molds **2** (2022) V2A16 https://doi.org/10.5281/zenodo.6762767

# Assessment of Natural and Synthetic Substrates as Spore Traps for Myxomycetes

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Received: 9 March 2022
Accepted for publication: 27 June 2022
Published: 30 June 2022
Corresponding editor: Steven L. Stephenson

**Abstract:** The presence of myxomycete spores in the air is undisputed; however, there are very few studies to identify substrates that can trap these airborne spores. In this study, we evaluated six substrates – three natural, three synthetically-made – for their ability to trap myxomycete spores. We tested our spore baits under open-spaced (rural) and enclosed or limited airflow (urban) settings. Our study identified eight species of myxomycetes, namely, *Arcyria cinerea* (Bull.) Pers., *Diderma effusum* (Schwein.) Morgan, *Diderma hemisphaericum* (Bull.) Hornem., *Didymium squamulosum* (Alb. & Schwein.) Fr. & Palmquist, *Perichaena depressa* Lib., *Perichaena* cf. *vermicularis* (Schwein.) Rostaf., *Physarum album* (Bull.) Chevall., and *Stemonitis fusca* Roth. Among the substrata, we observed the presence of myxomycetes mainly in spore baits with leaf litter regardless of exposure time or location. We did not observe myxomycetes in baits with coconut coir fibers and dried sphagnum moss while the baits with synthetic substrates showed myxomycetes, albeit with relatively very few numbers of records and species. The length of bait exposure did not impact the trapping of myxomycete spores. Our study evaluated for the first time the potential of synthetically made materials as spore traps for myxomycetes.

Keywords: airborne spores, baiting technique, moist chambers, spore trapping method



## Introduction

Spores in fungi as with other microorganisms serve as reproductive cells which germinate and develop into new individuals and facilitate colonization of new habitats and survival under harsh environmental conditions. Myxomycetes, also known as plasmodial slime molds, were once regarded as fungi due to their similarities in fruiting body morphology and in the ability to produce many spores. Myxomycete spores germinate into uninucleate, haploid, multiple, ameboflagellate stages, which later develop into the multinucleate, diploid, plasmodial stage (Clark and Haskins 2016). Wind typically disperses these minuscule spores, which have thick pigmented walls and necessitate water and suitable conditions to germinate (Clark and Haskins 2016).

Multiple vectors have been identified for short- and long-distance dispersal of spores. These included passive mechanisms such as wind and precipitation and active dispersal through insects and other

animals (Golan and Pringle 2017). Slime molds form fruiting bodies so spores can be actively ejected in the wind and/or with spore cases that are raised aloft so that their bases can conveniently adhere to substrates while facilitating spore dispersal. Myxomycete spores are good candidates for aerodynamic studies because they are virtually spherical and lack attachment features (Tesmer and Schnittler 2007). The powdery spore mass is released from an evanescent peridium that dismantles during maturity (Keller and Everheart 2010). Schnittler and Stephenson (2000) also showed that a modest breeze could move myxomycete spores more than one kilometer from their origin. Without a doubt, long-term spore dispersion is a significant aspect of the myxomycetes' evolutionary success (Tesmer and Schnittler 2007).

Moist chamber culture technique, originally designed to observe algae, has been extensively used to study myxomycetes whose fruiting bodies are too small to be seen or may not have yet formed during field collection. A moist chamber is often a petri plate or plastic container lined with moistened filter paper. Dead plant materials abundantly found in the field are valuable examples of organic-based matter which are typically used as substrates in moist chamber cultures (Snell and Keller 2003). In addition, myxomycetes have also been reported fruiting over inanimate objects, e.g., aquarium glass (Tamayama and Keller 2013), dead animal skull (Keller et al. 2008) and disposed old cloth and plastics (dela Cruz and Eloreta 2020). The prevalence of myxomycetes in these natural substrata, even on inanimate objects, is possible primarily due to the spores trapped on these materials. In this study, we tested whether synthetic materials and natural substrata could effectively trap airborne spores to document the presence of myxomycetes in indoor or outdoor environments. Findings from this study contribute to the understanding of spore dispersal of myxomycetes while evaluating several substrata as spore traps.

## Materials and methods

### *Study sites*

For this research, we have chosen three sites (one rural, two urban) for the setting up of the spore traps (Figure 1). **Site one**: an open-space area within rice fields in Barangay Dawis Sur, Zarraga, Iloilo Province (10°51’03.2” N, 122°36’38.49” E, 12 masl, representing rural area), **site two**: a two-storey apartment complex, in Barangay Culiat, Quezon City (4°40’09.37” N, 121°03’07.04” E, 49 masl, urban #1), and **site three**: a low-rise, five-storey condominium unit in Barangay San Agustin, Novaliches, Quezon City (14°44'11.8" N, 121°02'09.6" E, 60 masl, urban #2), all in the Philippines. Sites two and three represented an urbanized area and are located within the capital region of Metro Manila, a highly dense metropolis with a land area of 636 $km^2$ and a population of 13 million. The baits in the urban environment were setup in home balconies (approx. 5 $m^2$) located in the inner complex of the housing units, i.e., it faced another home units, and thus, are described as enclosed-space or with limited airflow area (Figure 1).

### *Spore baits*

Three natural substrates (dried sphagnum moss [MO], coconut coir [CC] fibers, and leaf litter [LL]), and three synthetic substrates (scouring pad [SP], faux fur cloth [FF], and polyester fiber [PF]), were used as spore baits in the study (Figure 2). Some of these substrates, namely LL, SP and FF, were cut into postage-stamp-sized (3 cm × 2 cm) pieces. These natural and synthetic materials were chosen for their dense, filament (hair-like), tangled networks that can easily trap spores and mimic the physical properties of some of the easily observed household materials such as clothing, wall decoration (tapestry),

fabrics, mats, and carpets which are potential microhabitats or substrata for myxomycetes. Leaf litter, i.e., dried, hanging leaves of the tropical plant *Artocarpus heterophyllus* Lam., served as the control substrate as aerial leaf litter is regarded as good spore trap for myxomycetes (Rincón-Marín et al. 2021) and as ideal substrate (microhabitat) for moist chamber cultures as shown in the studies of Alfaro et al. (2014), Stephenson and Landolt (2015), and Dagamac et al. (2017).

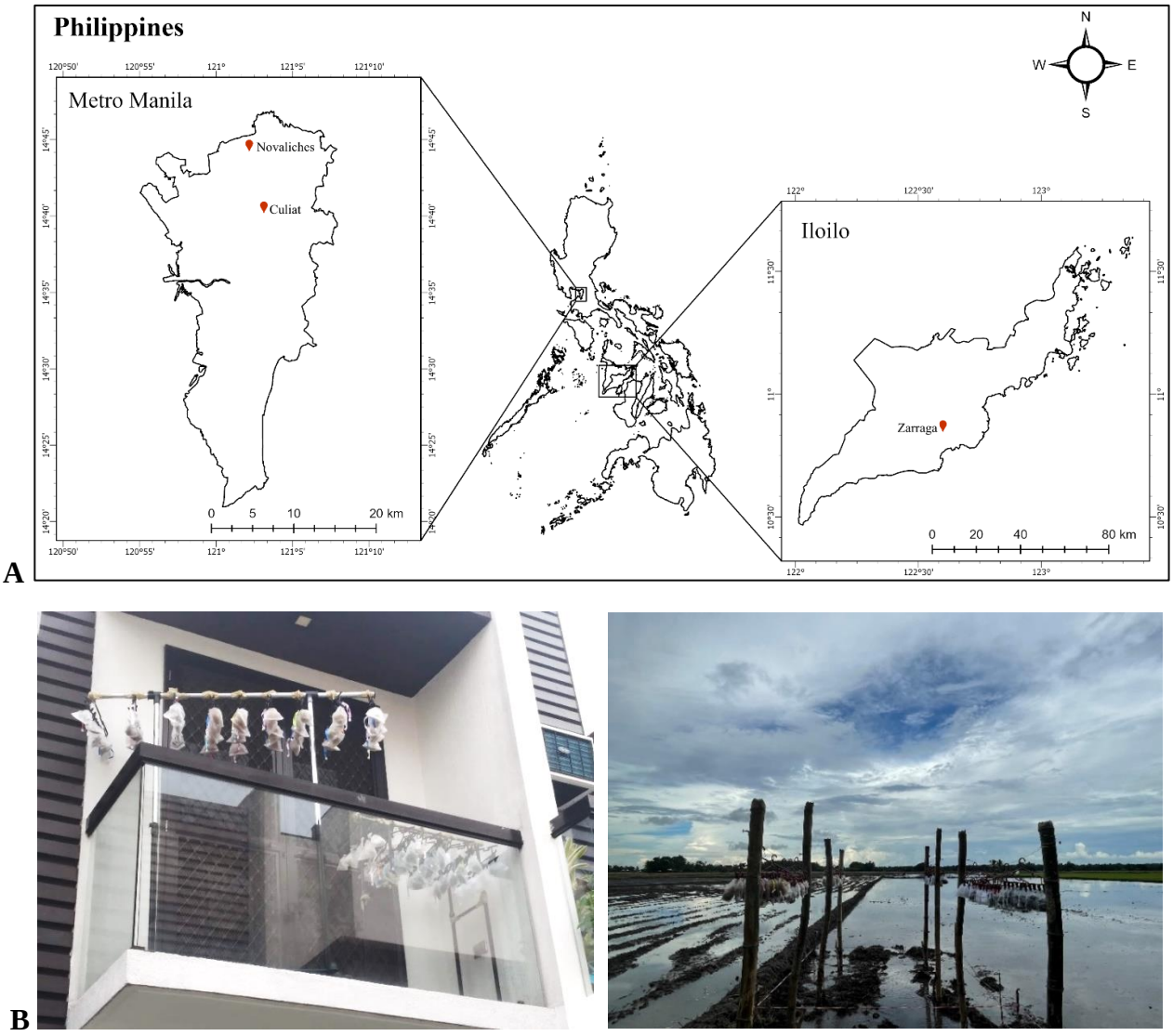


**Figure 1.** (A) Map and (B) images of the experimental sites representing urban (Metro Manila, lower left) and rural (Iloilo, lower right) settings.

All materials except leaf litter were purchased online. To ensure that spores that may be present on the natural substrates are not accounted in the study, the natural substrates were initially placed in an autoclavable plastic bag and sterilized with a commercially available home pressure cooker for 15 minutes. Swenson et al. (2018) confirmed the suitability of commercial electric pressure cookers in sterilizing culture media and laboratory items in the absence of a laboratory steam autoclave. They also stated that

pressure cookers can inactivate high titres of bacteria and fungi and can sterilize contents to a level that is acceptable for laboratory use. Due to the physical nature of the materials, the synthetic substrata were not sterilized. All substrata were individually placed in nylon mesh bag with a pore size of 2 mm and randomly hanged side by side with cloth hangers. Fifteen spore trap baits were prepared for each of the six substrates, with a total of 90 samples per study site. Each clothing hanger was placed on balconies for the urban sampling locations with an elevational range between 49 and 60 meters above sea level (masl) while the rural sampling site was in an open rice field of 12 masl. The spore baits were left undisturbed in the baiting areas, and then, three representative samples were taken consecutively after the 2$^{nd}$, 4$^{th}$, 6$^{th}$, 8$^{th}$, and 10$^{th}$ week of exposure. The collected baits were initially placed inside brown paper, stored in a dry area, and later used for the preparation of the moist chamber cultures after the last exposure period (i.e., 10$^{th}$ week). Exposure of the spore baits was from June to August 2021, which coincided with the rainy season in the Philippines.


**Figure 2.** Substrates utilized in the experiment: [A] Natural: (1) dried sphagnum moss, (2) coconut coir fiber, (3) leaf litter, and [B] Synthetic: (1) scouring pad, (2) faux fur cloth, and (3) polyester fiber.

*Moist chamber preparation, species identification, and data analysis*

A total of 270 moist chambers were prepared in this study following the standard protocol of Stephenson and Stempen (1994). The spore baits were placed inside disposable petri plates lined with paper towel, soaked overnight with distilled water, and subsequently, the pH was determined with a portable digital pH meter and the surplus water drained out. Ample amount of natural and synthetic substrata was added for fill in the petri plates (Figure 2). For the synthetic substrates, one piece of sterile oat flakes was added at the center of the moist chamber cultures to serve as nutrients for any colonizing microorganisms which can be feed upon by the growing slime molds. The moist chambers were incubated at room temperature (22–25 ºC) under diffuse light and examined for myxomycetes every day for the first

two weeks and then, once a week until the 12th week. The number of moist chambers positive for myxomycetes either as plasmodia and/or fruiting bodies were counted per exposure period/substrate types and used to determine the moist chamber productivity. One moist chamber positive for a species of myxomycete for each substrate type was considered as one positive collection or record for that species. Any visible fruiting bodies were collected in herbarium boxes, observed for their morphological traits, and identified using published literature (e.g., Stephenson and Stempen 1994; Keller and Braun 1999), and online ID guide (http://slimemold.uark.edu/). The taxonomic diversity index (TDI) was computed by dividing the number of recorded species by the number of the genera.

## Results

A total of 46 moist chambers, equivalent to 17% MC productivity, were positive for the presence of myxomycetes, either as plasmodia and/or fruiting bodies (Table 1). Moist chambers with leaf litter (LL) as baits were positive for all locations and collection times. The dried sphagnum moss (MO) and coconut coir (CC), despite being natural substrates, failed to show any growth of myxomycetes. The three synthetic-based substrates recorded positive moist chambers, albeit with a relatively low number of one to two moist chambers only. The number of successful baits was the same regardless of the sampling sites (open-spaced, rural setting vs. enclosed spaced, urban area), 15-16 positive moist chambers per site, owing to the very successful trapping of spores by the leaf litter baits. The exposure time of the spore baits did not influence the myxomycete yield even for leaf litter which showed positive results in almost all the moist chambers under all exposure period (Table 1).

Eight species of myxomycetes from six genera were collected successfully by the spore baits in this study (Table 2, Figure 3). All species were recorded from leaf litter as spore baits. Among the synthetic substrata, scouring pad captured spores of *Diderma hemisphaericum* while *Stemonitis fusca* grew on faux fur. One moist chamber with polyester fiber was positive for myxomycetes as plasmodium (Table 1) but failed to develop into identifiable fruiting bodies. Twenty six moist chambers with leaf litter were also positive for plasmodia that did not further develop into fruiting bodies.

Table 3 compares the moist chamber productivity and number of myxomycetes between the experimental stations. All three areas showed a very low MC productivity of 17-18% and expectedly, also a low number of fruiting body occurrences, only 28 recorded specimens or collections. The highest number of fruiting body occurrences was noted in the rural area where the spore baits were set up in an open-spaced rice field. The two urban sites representing enclosed area had a similar MC percent productivity, but with a much lower number of records, only four and six. A significantly high number of positive moist chambers, nine for Culiat (urban site #1) and 12 for Novaliches (urban site #2) in Quezon City, had plasmodia that failed to further develop into fruiting bodies. Only *Arcyria cinerea* and *Stemonitis fusca* were recorded from the moist chambers in urban areas as opposed to all eight species being recorded in the rural area.

## Discussion

Spore formation is a crucial stage in the life cycle of spore-bearing organisms like myxomycetes. The successful colonization of any substrata can be correlated with successful spore dispersal, but there are many factors that can affect this. The presence of myxomycete species is significantly influenced by

**Table 1.** Number of moist chambers positive for myxomycetes based on baits exposure time.

| Substrates[a] | | mean pH | Number of MC | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 | Number of positive MC |
|---|---|---|---|---|---|---|---|---|---|
| *Rural Area* | LL | 6.5± 0.25 | 15 | 3 | 2 | 3 | 2 | 2 | **12** |
| | MO | 5.3± 0.27 | 15 | - | - | - | - | - | **0** |
| | CC | 5.3± 0.27 | 15 | - | - | - | - | - | **0** |
| | PF | 6.4± 0.33 | 15 | - | - | - | - | - | **0** |
| | FF | 6.2± 0.16 | 15 | 2 | - | - | - | - | **2** |
| | SP | 7.2± 0.38 | 15 | - | - | 1 | - | - | **1** |
| *Urban Area #1* | LL | 6.5± 0.21 | 15 | 3 | 3 | 3 | 3 | 3 | **15** |
| | MO | 5.8± 0.11 | 15 | - | - | - | - | - | **0** |
| | CC | 5.5± 0.26 | 15 | - | - | - | - | - | **0** |
| | PF | 6.6± 0.27 | 15 | - | - | - | - | - | **0** |
| | FF | 6.0± 0.32 | 15 | - | - | - | - | - | **0** |
| | SP | 7.5± 0.20 | 15 | - | - | - | - | - | **0** |
| *Urban Area #2* | LL | 6.7± 0.28 | 15 | 3 | 3 | 3 | 3 | 3 | **15** |
| | MO | 5.7± 0.14 | 15 | - | - | - | - | - | **0** |
| | CC | 6.0± 0.33 | 15 | - | - | - | - | - | **0** |
| | PF | 6.6± 0.14 | 15 | - | 1 | - | - | - | **1** |
| | FF | 6.3± 0.20 | 15 | - | - | - | - | - | **0** |
| | SP | 7.3± 0.12 | 15 | - | - | - | - | - | **0** |

[a]Natural substrates: LL (leaf litter), MO (dried sphagnum moss), CC (coconut coir fiber); Synthetic substrates: PF (polyester fiber), FF (faux fur cloth), SP (scouring pad).

**Table 2.** The number of collections of myxomycetes on different substrates that functioned as spore traps.

| | Natural[a] | | | Synthetic[b] | | |
|---|---|---|---|---|---|---|
| | **LL** | **MO** | **CC** | **PF** | **FF** | **SP** |
| Total number of MC | 45 | 45 | 45 | 45 | 45 | 45 |
| **% MC productivity** | **93** | **0** | **0** | **0** | **4** | **2** |
| *Arcyria cinerea* | 12 | - | - | - | - | - |
| *Diderma effusum* | 4 | - | - | - | - | - |
| *Diderma hemisphaericum* | 2 | - | - | - | - | 1 |
| *Didymium squamulosum* | 1 | - | - | - | - | - |
| *Perichaena depressa* | 2 | - | - | - | - | - |
| *Perichaena* cf. *vermicularis* | 1 | - | - | - | - | - |
| *Physarum album* | 1 | - | - | - | - | - |
| *Stemonitis fusca* | 2 | - | - | - | 2 | - |
| **Total** | **25** | **-** | **-** | **-** | **2** | **1** |
| Number of records | 25 | - | - | - | 2 | 1 |
| Number of species | 8 | - | - | - | 1 | 1 |
| Number of genera | 6 | - | - | - | 1 | 1 |
| Taxonomic diversity index (S/G)[c] | 1.3 | - | - | - | 1 | 1 |

[a] Natural substrates: LL = leaf litter, MO = dried sphagnum moss, CC = coconut coir fiber
[b]Synthetic substrates: PF = polyester fiber, FF = faux fur, SP = scouring pad
[c]The lower is the TDI value, the more diverse is a particular biota.

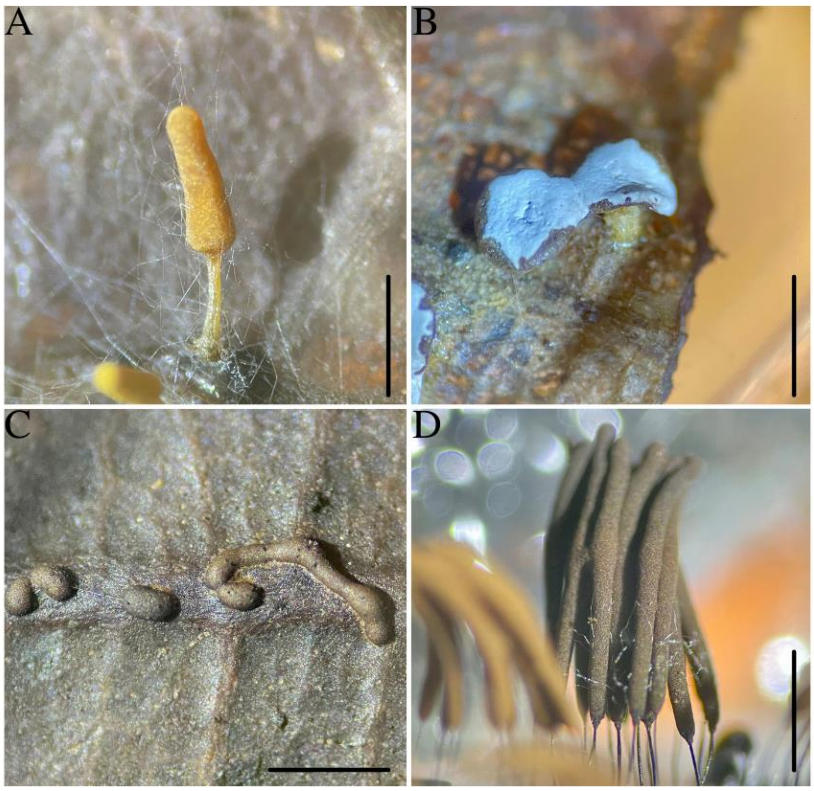


**Figure 3.** Representative myxomycetes recorded in this study. (A) *Arcyria cinerea* (Bull.) Pers., leaf litter natural substrate; (B) *Diderma hemisphaericum* (Bull.) Hornem., leaf litter natural substrate; (C) *Perichaena* cf. *vermicularis* (Schwein.) Rostaf., leaf litter natural substrate; and (D) *Stemonitis fusca* Roth, faux fur synthetic substrate. Scale bar = 1.5 mm.

**Table 3.** Moist chamber productivity and number of species recorded in the three study areas.

| | Rural Area (open-space) | Urban Areas (enclosed-space) | |
|---|---|---|---|
| | **Iloilo** (Zarraga) | **Quezon City** (Culiat) | **Quezon City** (Novaliches) |
| Number of MC | 90 | 90 | 90 |
| Percent (%) productivity | 17 | 17 | 18 |
| Total number of records | 18 | 6 | 4 |
| Number of species | 8 | 2 | 1 |
| Number of genera | 6 | 2 | 1 |
| Taxonomic diversity index (S/G) | 1.3 | 1 | 1 |

the pH-measured acidity of the substrate. Schnittler and Stephenson (2000) indicated that an increase in substrate pH was generally positively connected with an increase in myxomycete species diversity in both litter and bark. In this study, the substrates used for moist chambers had pH between 5.3 to 7.5. The occurrence of frequent heavy rain tends to washout the spores from both the aerially suspended substrates and the atmosphere (Oliveira et al. 2009). The study of Rojas et al. (2020) also supported this idea. In their study, the presence of water film in the sterile mesh bags which contain dried leaves and that were set-up on the grounds may washed-off myxomycete spores or propagules. Schnittler et al. (2015) also stated that

moist chamber cultures of substrates collected in regions with pronounced rainy seasons may fail to recover a part of the area-specific species diversity as perhaps is the case with our study as our spore baits were set up during the rainy season in the Philippines. Wind is another important factor which could influence spore dispersal. A gentle wind can carry myxomycete spores more than a kilometer distance (Stephenson et al. 2008). In the study of Policina and dela Cruz (2020a), cardinal direction had no influence in the species diversity of bark-dwelling myxomycetes. However, they noted a relatively higher number of recorded species at the south and west directions which they attributed to the wind effect as the study was conducted during the southwest monsoon season. The study of Cabutaje et al. (2021) also suggested the potential impact of strong winds brought about by typhoons to myxomycete spore dispersal and the increase in available plant materials for colonization after a typhoon. In this study, we compared two scenarios related to spore dispersal – an open-spaced area with unobstructed wind and an enclosed area with limited wind flow. Contrary to our expectations, we did not observe any significant differences between the sampling localities in terms of moist chamber productivity and number of recorded specimens with leaf litter as spore baits (Table 2). Initially, we expected the low and interrupted air currents in the urban setting could contribute to low moist chamber productivity as similarly noted by Rincón-Marín et al. (2021), but our study showed low number of positive moist chambers in all three sampling areas. Hence, our findings could only be explained by the inability of the different substrata to effectively trap myxomycete spores.

Myxomycetes have been recorded from various plant types, plant detritus, and vegetation in every terrestrial habitat investigated to date. A study by Alfaro et al. (2014) suggested that areas with increased plant communities are correlated with an increase in myxomycete species. Similarly, a study by Ing (1983) indicated that the availability of plant species in a specific area, which serve as substrates for the sporulation of myxomycetes, affects the presence of myxomycete species within the vicinity. Several plant-based substrates were already recognized for myxomycetes and have been successfully used to make moist chambers. These included aerial and ground leaf litter (Pecundo et al. 2017, 2021; Rincón-Marín et al. 2021), twigs (Macabago et al. 2010), barks (Schnittler et al. 2012; Policina and dela Cruz 2020a, 2020b; Lim et al. 2021), woody vines (Vlasenko et al. 2018; Isagan et al. 2020), ferns (Alfaro et al. 2014), and dung of herbivorous animals (Schnittler et al. 2015). Other substrates that have been reported in the Philippines includes grass litter (Carascal et al. 2017), dead inflorescences (Pecundo et al. 2017; dela Cruz et al. 2021), and coconut inflorescences (Cabutaje et al. 2021). Table 2 shows that spore baits with aerial leaf litter was the most successful in trapping myxomycete spores with 93% MC productivity. All eight (8) species reported in this study were observed from the said substrate. The study of Alfaro et al. (2014) in Negros Occidental, Philippines showed a 72% level of success for aerial leaf substrates while Macabago et al. (2010) recorded 85% MC productivity with the same substrate type. In the study of Pecundo and her colleagues (2021), aerial leaf litter and woody vines, which are examples of above-ground plant materials, have a greater MC productivity (78%) as compared to plant materials collected from the forest floor, i.e., twigs (72%) and ground leaf litter (63%). In the studies of Kuhn et al. (2013) and Redeña-Santos et al. (2017), aerial litter also revealed a higher number of species than ground litter. Such high productivity yield from aerial leaf litter was recorded from other studies in the tropics (Rojas and Stephenson 2008) and in the temperate region (dela Cruz et al. 2014). The leathery leaf structure of leaves can contribute to why leaf litter are very good spore traps for myxomycetes. In our study, we used the dried leaves of *Artocarpus heterophyllus* due to its thin-leathery texture. Hairy and fibrous leaf surfaces (e.g., of *Camellia sinensis* (L.) Kuntze) and thick leathery leaves (e.g., of *Dimocarpus longan* Lour. and *Psidium guajava*

L.) are favorable natural spore traps especially for those myxomycetes that produce spores with warts and spines ornamentation (Redeña-Santos et al. 2017).

Contrary to our expectations, no myxomycetes were recorded in the sphagnum moss substrates despite their seemingly good physical attributes for trapping spores (Stephenson and Rojas 2020). Coconut coir fiber also did not show any myxomycete growth, even though its physical property for trapping spores is similar to that of moss and myxomycetes have been reported from coconut substrata (Sá et al. 2022). Perhaps, for these natural substrates a longer exposure period is required to trap spores. On the other hand, it is also possible that myxomycete spores were trapped in these substrata but failed to germinate. Synthetic substrates with similar surface textures such as faux fur, scouring pad and polyester fiber did trap myxomycete spores, although not as efficient as the leaf litter substrates. Only *Diderma hemisphaericum* and *Stemonitis fusca* were observed from scouring pad and faux fur, respectively. Other studies have highlighted the ability of myxomycetes to emerge from uncommon substrates such as disposed old cloth (Ranade 2012), plastic pieces (dela Cruz and Eloreta 2020), pinecones attached to living trees (Keller et al. 2008), dead animal skull (Keller et al. 2008), and aquarium glass (Tamayama and Keller 2013). Ample nutrient supply is also a pre-requisite. For example, in an aquarium setting, myxomycetes submerged in water survive by feeding on diatoms and green algae (Tamayama and Keller 2013). In our study we added a piece of sterile oat flake to serve as source of nutrients for contaminating bacteria, to which myxomycetes feed upon. However, even such addition of a single sterile oat did not lead to higher myxomycete yield. Nevertheless, synthetic materials, while are not good to support growth of myxomycetes, can still capture myxomycete spores.

## Acknowledgments

The authors acknowledge the Department of Agriculture-Western Visayas (DA-WV), the Western Visayas Integrated Agricultural Research Center (WESVIARC), and the Regional Animal Disease Diagnostic Laboratory (RADDL), Iloilo City, specifically, Lerisa E. Balopeños and Czarina Kristine D. Nobleza, for the use of stereomicroscope in the confirmation of our species identities.

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