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PROTOCOL NOTE

Applications in Plant Sciences

An inexpensive moist chamber culture technique for finding microbiota on live tree bark

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Abstract

Premise: Traditional moist chamber cultures (MCs) prepared in aseptic laboratory environments using sterile Petri dishes are commonly used to quantify the microbiota of rough-bark tree species and woody vines. MCs are typically expensive and may be difficult to make, so a less expensive option made from easily available supplies was developed. These cost-friendly MCs were compared with standard laboratory methods to demonstrate their efficacy.

Methods and Results: Modified MCs were made using inexpensive, store-bought supplies; compared to a standard laboratory setting, the modified MCs are shown to be less expensive with a faster setup time and larger size that facilitates a variety of tree and woody vine species. MC use resulted in the discovery of new species of fungi and myxomycetes with associated locality records. We provide detailed instructions for creating modified MCs, as well as a list of myxomycete species and their associated bark characteristics, pH values, and water-holding capacity.

Conclusions: This new, low-cost MC technique makes the study of microbiota more inclusive and accessible for those in research laboratories, classrooms, and homes, including both amateurs and professionals. MCs are easy to prepare, versatile, and applicable for many areas of botany and the biological sciences, potentially allowing exploration into unexplored areas in urban ecosystems.

KEYWORDS

biodiversity, fungi, myxomycetes, slime molds, urban ecology, video

The first general use of moist chamber cultures (MCs) to isolate myxomycetes (plasmodial slime molds) from live tree trunk bark is attributed to G. W. Martin and H. C. Gilbert, working in the Mycological Laboratory of the Department of Botany at the University of Iowa (Gilbert and Martin, 1933; Gilbert, 1934). Rare and newly described species were included in their original list of myxomycetes, and more recently, additional new and rare species of myxomycetes have been found using the MC technique (Keller and Brooks, 1976, 1977; Keller, 2004; Keller et al., 2008, 2009; Keller and Marshall, 2019) as well as fungi (Perry et al., 2020) (Figure 1). Some of these species were discovered using a rope-climbing technique to collect bark samples for MC cultures in the tree canopy (Parker and Keller, 2003; Snell and Keller, 2003; Keller et al., 2004; Everhart et al., 2009; Kilgore et al., 2009; Scarborough et al., 2009). Collection techniques to obtain bark samples from trunks of live trees and woody vines for MC cultures are described in Keller and Braun (1999), Keller and Everhart (2010), and Keller et al. (2009). Examples of myxomycete species associated with the two tree species focused on here, *Juniperus virginiana* L. (eastern red cedar) and *Ulmus americana* L. (American elm), are provided in Tables 1 and 2.

As described by Stephenson (1982, 1985), laboratory preparation of MCs involved field collection of live tree trunk bark and ground samples of decayed wood and leaves. This methodology included sterile plastic Petri dishes, boiled tap water, and costly laboratory equipment, but the studies did not report detailed bark characteristics. In these

studies, only myxomycete species were targeted; moreover, little information was provided for trees (e.g., tree species designations, a complete list of rough-bark tree species, examples of smooth-bark unproductive tree species, pH of bark, myxomycete species associations, water-holding capacity of bark) and supply costs were not included.

FIGURE 1 Fleshy mushroom *Mycena ulmi* growing on the surface of live *Ulmus americana* tree bark in a moist chamber culture. (Photo by Brian A. Perry, reproduced with permission from the *Journal of the Botanical Research Institute of Texas*; Perry et al., 2020).

In contrast, the new MC culture technique described here can recover a broader spectrum of species at a much lower cost. This MC technique found diverse organisms on live tree bark, including green algae, cyanobacteria (blue-green algae), myxobacteria, fungi (molds and fleshy mushrooms), lichens (crustose and foliose types), liverworts, mosses, nematodes, tardigrades, and insects; the presence of fungi, myxomycetes, nematodes, and tardigrades demonstrates that this technique has broad use for the botanical research community and biologists. By using low-cost store-bought supplies available from local sources and a low-tech approach, this MC technique is inclusive of a broader spectrum of users, including research and amateur botanists, classroom teachers, and community scientists. The design of this modified MC still serves the same purpose as the traditional MC—to create ideal conditions in a microenvironment to facilitate the formation of fungi and myxomycetes, while allowing safe observation over a longer time.

There is a growing need for more cost-effective accessibility in research laboratories and increased involvement of community scientists in scientific research. Many researchers may be in academic or private institutions where funding is lacking or inadequate, physical access to research laboratories may not be possible, or informed

TABLE 1 Myxomycete species found on live *Juniperus virginiana* tree bark, arranged by taxonomic order, genus, and species. Nomenclature generally follows Martin and Alexopoulos (1969).[a]

	Order				
	Echinosteliales	**Liceales**	**Physarales**	**Stemonitales**	**Trichiales**
Field collections	*Clastoderma debaryanum* var. *emperatorium* *C. microcarpum* *C. pachypus* *Echinostelium arboreum* *E. coelocephalum* *E. elachiston* *E. fragile* *E. minutum*	*Cribraria minutissima* *C. violacea* *Dictydiaethalium plumbeum* *Licea biforis* *L. denudescens* *L. inconspicua* *L. kleistobolus* *L. operculata* *L. parasitica* *L. pedicellata* *L. pseudoconica* *L. scyphoides*	*Badhamia affinis* *B. rugulosa* *Badhamiopsis ainoae* *Diachea arboricola* *Diderma corrugatum* *D. chondrioderma* *Didymium clavus* *D. orthonemata* *D. synsporon* *Physarum auriscalpium* *P. crateriforme* *P. nutans* *Trabrooksia applanata*	*Comatricha laxa* *Macbrideola cornea* *M. declinata* *M. decapillata* *M. scintillans* *Stemonitis flavogenita*	*Arcyria cinerea* *Calomyxa metallica* *Minakatella longifila* *Perichaena chrysosperma* *P. depressa* *P. minor* *P. minor* var. *pardina*
Moist chamber cultures (Traditional method)	*Clastoderma debaryanum* var. *emperatorium* *C. microcarpum* *C. pachypus* *Echinostelium arboreum* *E. coelocephalum* *E. elachiston* *E. fragile* *E. minutum*	*Cribraria minutissima* *C. violacea* *Licea biforis* *L. denudescens* *L. inconspicua* *L. kleistobolus* *L. nannengae* *L. operculata* *L. parasitica* *L. pedicellata* *L. pseudoconica* *L. scyphoides*	*Badhamia affinis* *B. rugulosa* *Badhamiopsis ainoae* *Diachea arboricola* *Didymium clavus* *Physarum auriscalpium* *P. crateriforme* *P. nutans*	*Comatricha laxa* *Macbrideola cornea* *M. declinata* *M. decapillata* *M. scintillans* *Stemonitis flavogenita*	*Arcyria cinerea* *Calomyxa metallica* *Minakatella longifila* *Perichaena chrysosperma* *P. depressa* *P. minor* *P. minor* var. *pardina*

[a]Total number of different myxomycete taxa from bark of living *Juniperus virginiana* trees = 54. Author citations can be found in Appendix S3.

TABLE 2 Myxomycete species found on live *Ulmus americana* tree bark, arranged by taxonomic order, genus, and species. Nomenclature generally follows Martin and Alexopoulos (1969).[a]

	Order				
	Echinosteliales	**Liceales**	**Physarales**	**Stemonitales**	**Trichiales**
Field collections	*Clastoderma debaryanum* var. *emperatorium* *C. microcarpum*	*Cribraria confusa* *C. minutissima* *C. violacea* *Licea denudescens* *L. inconspicua* *L. kleistobolus* *L. marginata* *L. nannengae* *L. operculata* *L. parasitica* *L. pedicellata* *L. scyphoides*	*Badhamia affinis* *Badhamiopsis ainoae* *Diderma chondrioderma* *D. corrugatum* *Didymium clavus* *D. orthonemata* *Physarum crateriforme* *Trabrooksia applanata*	*Comatricha cornea* *C. laxa* *Macbrideola cornea* *M. decapillata* *Stemonitis flavogenita*	*Arcyria cinerea* *Calomyxa metallica* *Dianema* (clustered spores) *Minakatella longifila* *Perichaena chrysosperma* *P. depressa* *P. quadrata*
Moist chamber cultures (Traditional method)	*Clastoderma debaryanum* var. *emperatorium* *Echinostelium arboreum* *E. coelocephalum* *E. minutum*	*Cribraria confusa* *C. minutissima* *C. violacea* *Licea denudescens* *L. inconspicua* *L. iridescens* *L. kleistobolus* *L. nannengae* *L. operculata* *L. parasitica* *L. pedicellata* *L. pseudoconica* *L. scyphoides*	*Badhamiopsis ainoae* *Didymium clavus* *Physarum crateriforme*	*Comatricha cornea* *C. fimbriata* *C. laxa* *Enerthenema papillatum* *Macbrideola cornea* *M. decapillata* *M. scintillans* *Stemonitis flavogenita*	*Arcyria cinerea* *Calomyxa metallica* *Minakatella longifila* *Perichaena chrysosperma* *P. depressa* *P. quadrata* *P. minor*

[a]Total myxomycete species on *Ulmus americana* = 42. Author citations can be found in Appendix S3.

expertise to identify taxa is not available. This updated do-it-yourself moist chamber culture was designed to be done with inexpensive, cost-effective, readily available, and time-saving supplies that are available in both research laboratories and home settings to facilitate the observation, collection, and publication of microbiota data.

METHODS

Cost comparisons and overview of moist chamber culture preparations

Researchers studying fungi and myxomycetes in the early 1930s used standard (and more expensive) laboratory equipment, such as sterile glass Petri dishes, to make moist chamber cultures. More recently, sterile disposable plastic Petri dishes have become more common in modern laboratories. These Petri dishes and sterile deionized water have contributed in part to repeatability within the controlled environmental parameters of MCs (Figure 2A). However, there are disadvantages to using this standard laboratory equipment. These supplies are typically available only in larger bulk lots, leading to overall higher costs and larger space requirements for storage; furthermore, Petri dishes have size limitations of about 150 mm in diameter and 15 mm in depth, which does not allow the use of larger or greater numbers of tree bark pieces (size often limited to 4–5 smaller bark pieces). A standard reusable aluminum foil pie tin was chosen to replace the traditional Petri dish due to its wide availability, lower cost, and larger size (Figure 2B). This pie tin, about 25 cm in diameter and >2.5 cm in depth, permits the use of more pieces (8–12 larger bark pieces without overlapping) as well as thicker tree bark sizes, thus increasing the possibility for multiple microbiota in shorter setup times. This modified methodology resulted in cost and time savings (Table 3); furthermore, pie tin culture containers can be washed with soap and water, sterilized with alcohol, dried, and reused, thus reducing the cost even more. One disadvantage of this MC protocol is that it is more susceptible to airborne contaminants because of less sterile conditions; however, this can be minimized by scanning observations through clear plastic wrap and limiting the amount of uncovered exposure time to external surroundings.

These batch cultures have resulted in harvesting many tiny myxomycete species (100–200 μm), especially in the genera *Echinostelium* and *Licea*, or slightly larger sizes for *Macbrideola* species (Figure 3), enabling many more myxomycete fruiting bodies per collection deposited in the herbarium of the Botanical Research Institute of Texas (BRIT). This modified MC culture was developed at BRIT and has been used in published papers (Keller and Marshall, 2019; Perry et al., 2020). Proper curation and labeling become extremely important, especially when describing species that are new to science (Figure 4).

Here, we provide a brief summary of the MC preparation instructions; the detailed protocol is provided

in Appendix S1. Using rough bark samples from living trees and woody vines (Table 4) increases the chances of harvesting myxomycetes and fungi. *Juniperus virginiana* tree trunk bark, shown as an example in Video S1, can often be removed using fingers to strip the bark. Low-cost materials used for the batch MCs can be purchased at local stores; these include reusable pie tins, paper towels, mineral spring or distilled water, and clear plastic wrap for quick preparation and wetting bark samples. Periodic examination of MC cultures over several weeks using a dissecting microscope at magnifications of 20–100× reveals an assortment of life forms. Proper curation and deposition of specimens is essential; therefore, as an example, we provide here instructions from BRIT for harvesting, boxing, and labeling specimens for transport (Appendix S2). A link to a Google Form is also available on the BRIT website for the public to submit questions and their own MC images to the program staff to answer and review.

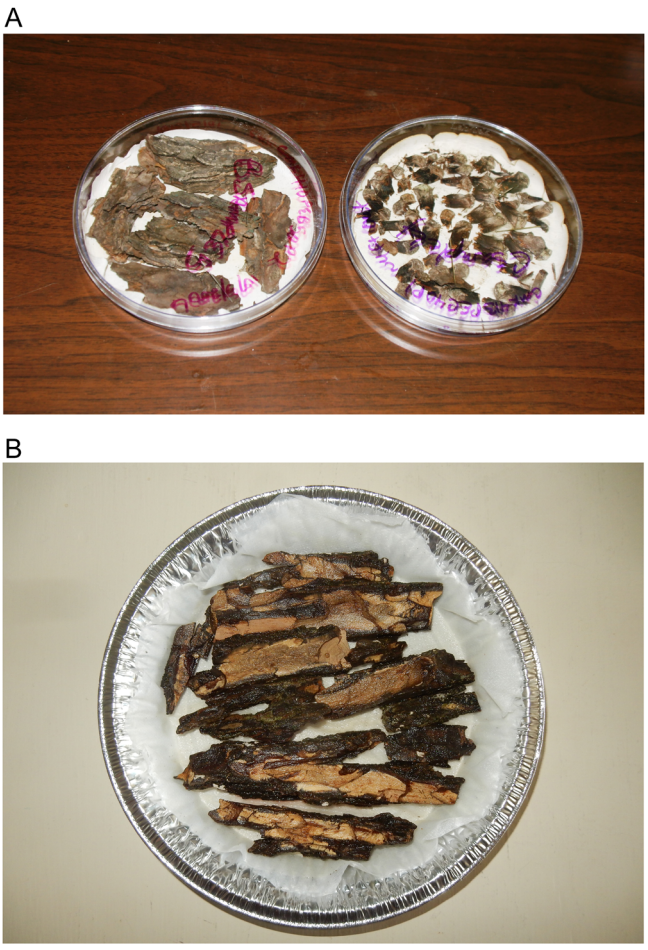

FIGURE 2 Traditional and modified moist chamber culture (MC) techniques. (A) Traditional MC technique using sterile oversized plastic disposable Petri dishes. (Photo by Courtney M. Kilgore, used with permission.) (B) Uncovered and reusable foil pie tin used for batch MC technique. (Photo by Bob O'Kennon, used with permission.)

Visual and instructional aids

Materials and methods to make traditional MCs are available in publications that may or may not be freely accessible online, and there has never been an accompanying online visual aid for the process of creating any MC. To make this new MC method more useful and accessible, we have created a step-by-step instructional video (Video S1); this is also available on YouTube (https://youtu.be/aiwUkbz947M) and on the Fungi, Myxomycetes, and Trees Research Program webpage of the Fort Worth Botanic Garden-BRIT website (https://fwbg.org/research-projects/fungi-myxomycetes-and-trees-program/diy-moistchamber/). Detailed instructions accompanying this video are provided in Appendix S1 and are also available as a PDF download at https://fwbg.org/wp-content/uploads/2021/06/FINAL-DIY-Moist-Chamber-Culture.pdf.

The four-minute video begins by highlighting the materials needed to create the MC, as well as additional materials needed for collecting a tree bark sample. The video then shows how to collect bark from a live tree, gives examples of the data to be collected, and explains each step of constructing a MC. The video concludes with compound microscope images showing examples of the early life-cycle stages of myxomycetes that begin to emerge within the first days following the first wetting of the MC (Figure 3). Next, examples of other microbiota that may be seen in a MC, such as mosses, insects, and nematodes, are shown; the video concludes with images of common contaminants of

TABLE 3 Cost comparison of moist chamber cultures (MC).[a]

Traditional MC		**Modified MC**	
Materials	**Price (USD)/unit**	**Materials**	**Price (USD)/unit**
Plastic, sterile, disposable Petri dish bottom with top lid	$2.24 to $4.55	Reusable aluminum foil pie tin	$0.40 to $1.25
		Plastic sheet PVC cling film wrap	$0.013 per sq. ft.
Deionized and sterilized water (30 mL)	$5.40	Non-sterile natural spring water (30 mL)	$0.08
Sterile Whatman P8-creped filter paper (150 × 25 mm)	$0.13	Non-sterile white paper towels	$0.08
Fine-point permanent marker	$1.00	Fine-point permanent marker	$1.00
Total cost	**$8.77 to $11.08**		**$1.58 to $2.42**

[a]Material unit costs were based on estimates from scientific supply sources and local convenient hardware and grocery stores available in urban areas.

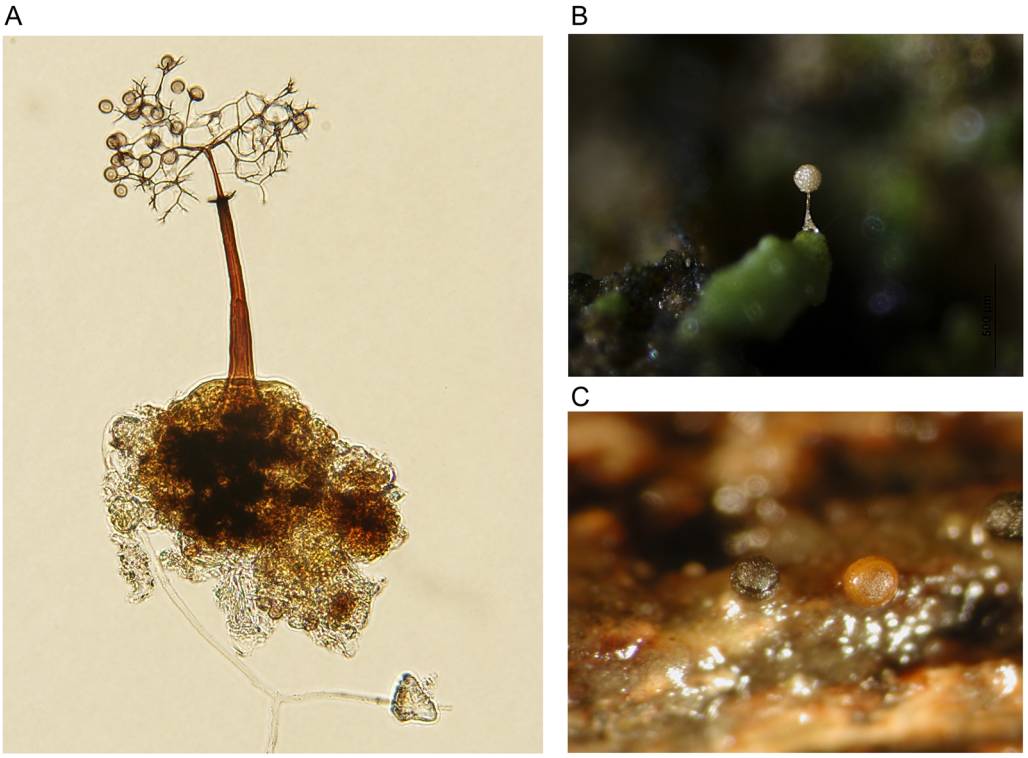

FIGURE 3 Examples of myxomycete species found in moist chamber cultures (MCs). (A) The stipitate sporangium of *Macbrideola cornea* (0.6–2.5 mm tall) is frequently found in MCs of live *Juniperus virginiana* and *Ulmus americana* tree bark. Note the translucent stalk, peridial collar, and branching capillitium. (Photo by Edward D. Forrester, used with permission.) (B) Stalked sporangium of *Echinostelium arboreum* (100–150 μm tall) showing persistent, shiny peridium on moss phyllid from the bark of a live *Ulmus americana* tree. (Photo by Edward D. Forrester, used with permission.) (C) Sessile sporangia of immature (right, orangish) and mature (left, blackish) *Licea parasitica* with operculum showing the circumscissile line of dehiscence (50–200 μm in diameter). This species is often found on *J. virginiana* and *U. americana* bark surfaces of live trees in association with crustose lichens. (Photo by Sydney E. Everhart, used with permission.)

MCs, such as types of green mold (species of *Trichoderma*) that may occur from long exposure to the air. There have been more than 800 views of this demonstration video since it was made public on 5 June 2021.

RESULTS

Examples of microbiota found on rough bark of live trees and woody vines

Live *Malus domestica* (Suckow) Borkh. (apple trees) planted in apple orchards, especially older abandoned orchards, are productive habitat sources for myxomycetes (Keller and Braun, 1999). Bark surfaces of mature, larger apple trees are roughened, irregularly fissured, sometimes thin, scaly, and peeling with myxomycete fruiting bodies often found on the underside of sloughing thin bark sections. Abundant sporangia of *Licea biforis*, *Perichaena depressa*, and *P. quadrata* (Figure 5) were found on large trunks of live apple trees in apple orchards and also developed in moist chamber cultures (Keller and Eliasson, 1992; Keller and Braun, 1999); additional rough bark tree species are listed in Table 4. These rough bark tree and woody vine species have in common bark characteristics that are mostly thicker, fissured, ridged, and sometimes cracking into squares or smaller scales that often create flowage waterways, with highly water-absorptive bark (Table 5) and a pH nearer a neutral 7.0 (Table 4).

Smaller, younger, and less mature trees are not as productive for microbiota as older, much larger trees (taller than 15 m and approximately 1.8 m in diameter). Bigger and older live trees are better for greater microbiota species diversity (Snell and Keller, 2003; Keller et al., 2009). Bark samples taken from about 2 m high on the tree trunk and on any side of the tree—especially along flowage waterways, deep fissures, and crevices—stay wetter longer and are more productive for myxomycete species. Lichens, mosses, or liverworts may be visible on bark surfaces; therefore, bark should be collected in these areas as well as in different locations on the tree to increase the probability for recovery of myxomycetes. Although tree bark is considered nonliving, the underlying tissues are living, so great care must be taken to avoid injuring trees. The size of bark samples should also be considered; pie-tin batch MC containers allow the use of bark pieces 15–23 cm, increasing opportunities for species abundance, as opposed to using plastic Petri dishes, which limit the size of bark pieces to 2.5–5 cm.

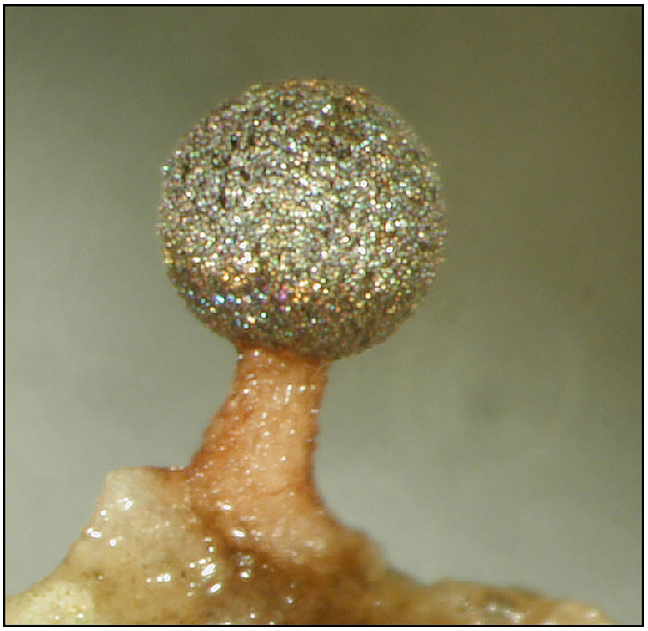

FIGURE 4 A stalked *Diachea arboricola* sporangium harvested from a MC. Note silvery iridescent peridial surface; additional developmental stages and scanning electron microscope images of this species are available in its original publication (Keller et al., 2004). This species was found on trunk bark of live *Quercus alba* and *Juniperus virginiana* trees. (Photo by Kenneth L. Snell, used with permission).

Herbicolous myxomycetes discovered on prairie plants using MC cultures

Herbicolous myxomycetes also have been discovered on vascular prairie plants. Samples of mature fruits and stems of herbaceous prairie plants harbor diverse and abundant myxomycete species (Table 6) (Kilgore et al., 2009). Prairie forbs like *Echinacea* species (prairie cone flower), *Yucca* species (Spanish bayonet), and *Asclepias syriaca* L. (common milkweed) can be found in native prairies, planted as ornamentals, and along roadside right of ways.

DISCUSSION

Environmental factors affecting microbiota on live tree bark

Bark pH is one factor that influences the presence, growth, and development of corticolous myxomycetes and fungi in MCs (Snell and Keller, 2003; Everhart et al., 2008; Scarborough et al., 2009; Kilgore et al., 2009; Perry et al., 2020). Therefore, during live tree bark collection, collectors should be aware that pH ranges and assemblages of acidophilic, neutrophilic, and basophilic myxomycete species associated with live tree species bark and woody vines increase the chances of finding a more diverse group of myxomycete species (Everhart et al., 2008, 2009; Kilgore et al., 2009; Scarborough et al., 2009). For example, *Cercis canadensis* L. (redbud) is a smaller tree with a higher pH value that also had a different composition of myxomycete species than the other prairie plants studied (Table 4) (Kilgore et al., 2009). This pH was measured by saturating the bark with neutral sterile water for 24 hours, then decanting the water and measuring the pH using a standard laboratory pH meter. More details regarding this method are available in Snell and Keller (2003).

FIGURE 5 *Perichaena quadrata* sessile sporangia growing in MC culture. (Photo by Sydney E. Everhart, used with permission).

It should also be noted that water-holding capacity (WHC) and retention enhance the presence, growth, and development of myxomycetes and fungi in MCs (Snell and Keller, 2003). The WHC of living tree bark has been determined for only five tree species: *Fraxinus americana* L., *Quercus alba* L., *Liriodendron tulipifera* L., *Acer rubrum* L., and *Pinus strobus* L. (Snell and Keller, 2003). The only significant difference in WHC was between the species that held the most (*L. tulipifera*) and the least (*P. strobus*) water (Table 5). This difference can be determined in part by sight because *L. tulipifera* bark initially absorbs more water in the MC culture dish and has a water-soaked appearance. In contrast, *P. strobus* has resiniferous bark, with beaded water droplets appearing on bark surfaces, so that more free water remains in the bottom of the Petri dish. Bark WHC was determined by measuring the difference in weight between the water-saturated bark and the oven-dried bark. More details can be found in Snell and Keller (2003).

Corticolous myxomycetes-associated trees and vines

Unfortunately, live tree bark examples of *Juniperus virginiana* and *Ulmus americana* were not part of the tree species test group for WHC (Snell and Keller, 2003). However, direct observation of MC cultures clearly showed both of these tree species have bark that acts like a wick and absorbs water quickly, resulting in a water-soaked

TABLE 4 List of rough bark live tree and woody vine species, with pH and number of myxomycete species found.[a,b]

Species name and authority	Common name	pH ± SE	No. of myxomycete species present
Malus domestica (Suckow) Borkh.	Domestic apple		10
Picea rubens Sarg.	Red spruce	3.7 ± 0.05	10
Pinus strobus L.	White pine	3.8 ± 0.16	24
Pinus echinata Mill.	Shortleaf pine	3.9 ± 0.88	14
Abies fraseri (Pursh) Poir.	Fraser fir	4.1 ± 0.06	none
Tsuga canadensis (L.) Carrière	Eastern hemlock	4.1 ± 0.08	17
Acer rubrum L.	Red maple	4.7 ± 0.14	49
Liriodendron tulipifera L.	Yellow poplar	4.9 ± 0.22	39
Taxodium distichum (L.) Rich.	Bald cypress	4.9 ± 0.24	20
Platanus occidentalis L.	American sycamore	5.1 ± 0.04	10
Vitis aestivalis Michx.	Summer grapevine	5.2 ± 0.03	15
Vitis vulpina L.	Fox grapevine	5.5 ± 0.08	12
Acer saccharum Marshall	Silver maple	5.5 ± 0.05	17
Quercus alba L.	White oak	5.7 ± 0.46	41
Liquidambar styraciflua L.	Sweet gum	5.8 ± 0.04	10
Cercis canadensis L.	Eastern redbud	6.3 ± 0.51	11
Tilia americana L.	American basswood	6.6 ± 0.08	10
Fraxinus americana L.	White ash	6.9 ± 0.16	31
Ulmus americana L.[c]	American elm	7.0 ± 0.12	42
Juniperus virginiana L.[c]	Eastern red cedar	7.0 ± 0.09	54

[a]Tree and woody vine species ordered from acidophilic pH (3.7–4.1), to mesophilic pH (4.7–5.8), to neutrophilic pH (6.3–7.0).

[b]Data adapted in part from the following publications: Keller and Braun (1999), Snell and Keller (2003), Keller (2004), Everhart et al. (2008), Keller et al. (2009), Kilgore et al. (2009).

[c]Species compiled from field-collected myxomycetes on the bark surface of living *Juniperus virginiana* and *Ulmus americana* trees and fruiting bodies harvested from moist chamber bark cultures. These myxomycete species were collected over a period of 35 years from approximately 250 trees. Bark was collected from these two tree species at a height of 1.5–2 m.

TABLE 5 Percent water-holding capacity (WHC) by tree and tree species.[a]

	Tree species				
Tree no.	*F. americana*	*Q. alba*	*L. tulipifera*	*A. rubrum*	*P. strobus*
1	123	65	107	105	84
2	120	49	141	132	53
3	51	114	146	103	60
4	60	80	117	83	80
5	82	90	68	118	55
Mean	87	80	116	108	66
SD	±33	±25	±31	±18	±15

[a]Data adapted from Snell and Keller (2003).

appearance. Rough bark surfaces, bark with higher WHC, and bark with a near-neutral pH clearly account for the higher species diversity of myxomycetes (Tables 4 and 5) (Snell and Keller, 2003; Scarborough et al., 2009).

Juniperus virginiana bark is thin, fibrous, flattened, with slight grooves or vertical crevices (Figure 6A). This shreddy bark can be peeled using fingers into long, thin strips sometimes 60–122 cm in length. The bark is often an ashy

TABLE 6 Prairie plant species, with pH and number of myxomycete species found.[a]

Species name	Common name	pH	No. of myxomycete species present
Yucca spp.	Great plains yuccas	7.1 ± 1.0	11
Echinacea spp.	Prairie coneflowers	7.3 ± 1.0	7
Asclepias syriaca	Common milkweed	7.9 ± 1.1	5

[a]Alkaliphilic pH rank ordered (7.1–7.9).

FIGURE 6 Rough bark surface characteristics of live (A) *Juniperus virginiana* and (B) *Ulmus americana* trees. (Photos by Bob O'Kennon, used with permission).

gray color on exposed surfaces and may have foliose or crustose lichens, leafy liverworts, mosses, or be void of epiphytes and bare. This bark acts like a wick with a higher WHC, which likely accounts in part for its higher myxomycete species diversity, often with hundreds of myxomycete fruiting bodies and sometimes up to six or eight species on a single tree. This bark makes ideal MC cultures because the thinner bark will fit evenly into the bottom of a culture pie tin. Many more generalist myxomycete species with pH values near or at pH 7 are found in great abundance on live *J. virginiana* trees (see Table 1) (Scarborough et al., 2009).

A species list of myxomycete species was compiled from field-collected myxomycete species on the bark surface of living *Juniperus virginiana* trees and fruiting bodies harvested from traditional moist chamber bark cultures (Table 1); this list was made over a period of at least 35 years from approximately 250 trees. Bark from most of these trees was collected at a height of 1.5–2 m. Two notable tree species included in our studies are widely available to collectors in our sample areas: *J. virginiana*, which was found in cemeteries, around rural homesteads, in shelter belts, along road right of ways and fence lines, and in open field habitats, and *Ulmus americana*, which is an ornamental shade tree found in residential areas and along streets and avenues in urban areas. Users may wish to search rough bark tree species in their home areas to discover myxomycete taxa anywhere and at any time.

Species of woody vines characterized by the genus *Vitis*, especially *V. aestivalis* Michx. and *V. vulpina* L., have grooved bark often peeling in long shredded strips that is notably water absorptive and productive for myxomycete species (Table 4).

Collecting bark from the trees and woody vines discussed above will maximize the chances for discovery and recording of myxomycete species throughout the midwestern, eastern, and southern USA. Many of the smaller myxomycete species in the genera *Echinostelium* and *Licea* are found on the trunk bark of living trees (Keller and Brooks, 1976, 1977; Keller and Braun, 1999; Keller and Marshall, 2019), and all five myxomycete orders are found on live tree and woody vine species. The following species listed in Tables 1 and 2 can be picture-keyed and easily recognized based on macromorphological features: *Arcyria cinerea*, *Cribraria violacea*, *Licea*

operculata, *L. parasitica*, *L. pedicellata*, *L. pseudoconica*, *Macbrideola cornea*, *M. decapillata*, *Perichaena chrysosperma*, *P. depressa*, *Physarum crateriforme*, *Stemonitis flavogenita*. They occur frequently on live tree bark samples in moist chamber cultures.

Smooth bark live trees unproductive for myxomycetes

Trees with smooth bark surfaces usually have thinner bark that adheres tightly to the underlying tissues. This bark is difficult to remove safely without injury to underlying tree tissues. Species of *Celtis* (hackberry) are good examples of this, with a bark surface that is smooth, thin, warty, and tight (e.g., *C. occidentalis* L. and *C. laevigata* Willd.). Species of birch can have peeling bark (e.g., *Betula cordifolia* Regel [heart-leaf paper birch]) and should be avoided. *Populus tremuloides* Michx. (quaking aspen) has smooth bark, often flaking or peeling from the woody core. *Fagus grandifolia* Ehrh. (American beech) also has smooth bark that is dense, tight, and difficult to sample without injuring the tree. Bark from older, larger *Platanus occidentalis* L. (American sycamore or planetree) trees originating from about 3–6 m into the upper canopy is often found peeled and broken into plate-like scales in sections on the ground. Samples collected nearer to ground level are more productive, whereas those collected at higher levels are unproductive. *Quercus* species (oaks) are usually productive for myxomycete fruiting bodies, but large red oaks (*Quercus rubra* L.) have dense, iron-like tight bark that is difficult to remove without injuring the tree. Individual trees may be exceptions, especially with abundant epiphytes of lichens, mosses, and liverworts, but in general this group of smooth bark tree species should be avoided for bark sampling.

CONCLUSIONS

The low-cost, low-tech, modified moist chamber culture technique described here (Appendix S1, Video S1) will make the study of microbiota accessible to researchers in science laboratories; community scientists; classroom teachers in elementary, middle, and high schools; and naturalists interested in exploring new life forms on the trunk bark of urban tree species. The cost comparison outlined here clearly shows that the modified batch MC method using reusable pie tins versus standard laboratory disposable plastic Petri dishes saves time and money. Myxomycete-productive tree species can be easily found in urban and residential environments. A list of 18 species of rough bark trees and two species of woody vines (Table 4) is provided here as possible collection sites for moist chamber cultures. The physical and chemical properties of these species are also provided to increase the chances of recovery of myxomycetes and fungi in moist chamber cultures. This new batch MC technique will allow professional and amateur researchers to discover many myxomycete and fungi taxa from their immediate environments.

AUTHOR CONTRIBUTIONS

A.P.B conceived of this research project by preparing the video and posting it on the BRIT website; all authors contributed to the video content and instructions. A.R.S. conducted the research using moist chamber cultures and collected and analyzed data with the support of H.W.K. A.P.B and H.W.K. prepared the original manuscript draft, and H.W.K. prepared the figures. A.R.S. and H.W.K. revised and edited manuscript drafts according to reviewers' suggestions. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

All data used in this study are found within this article and supplementary materials.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Detailed instructions to create a moist chamber culture and prepare collection data, to accompany Video S1.

Appendix S2. Information needed for myxomycete and fungal specimen labels for deposit in BRIT herbarium.

Appendix S3. Binomial names and author citations of the myxomycete species discussed. (+) represents laboratory observations made in moist chamber cultures. (x) represents field observations made on trunk bark of living trees.

Appendix S4. Myxomycete life cycle stages.

Video S1. How to create a moist chamber culture to view the biodiversity growing on live tree bark.

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