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Importance of Myxomycetes in Biological Research and Teaching

Harold W. Keller
University of Central Missouri, Warrensburg, haroldkeller@hotmail.com

Sydney E. Everhart
University of Nebraska-Lincoln, everhart@unl.edu

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Harold W. Keller[1] *and Sydney E. Everhart*[2]

Abstract

Myxomycetes, the true slime molds, are highlighted in research and teaching that emphasizes various stages of the life cycle as experimental models. Past and current phylogenetic classifications of Myxomycetes on the tree of life are presented. Life cycle stages are illustrated, described, and discussed. Simple laboratory demonstrations and experiments are described that include spore germination, spore release, and moist chamber cultures utilizing organic matter from various microhabitats. Novel compounds isolated from fruiting bodies and plasmodia of 22 myxomycete species are tabulated, some of which exhibit biological activity that function as antibiotics, antimicrobials, and are cytotoxic to cancer cells. Aeroallergens include myxomycete spores, especially *Fuligo septica*. The plasmodial stage of *Physarum polycephalum* has been used as a model research system to study responses to gravity in outer space, solve the shortest pathway through a maze exhibiting "primitive intelligence," develop a biologically controlled robot, discover what controls synchronous nuclear division, and the development of a new drug Polycefin that shows promise in the treatment of breast and brain cancerous tumors. Life span and senescence experiments showed that aging and longevity were under nuclear control. Environmental ground pollution may be remediated by myxomycete fruiting bodies and plasmodia of *Fuligo septica* that hyper-accumulate and concentrate highly toxic levels of zinc several thousand fold greater than site vegetation and lesser significant amounts of barium, cadmium, iron, manganese, and strontium. Tree canopy research has shown that aerial pollution results in the decrease of myxomycete species richness at higher elevations for *Abies fraseri* in Great Smoky Mountains National Park. At lower elevations and locations in the United States of America living *Juniperus virginiana* tree canopies have the highest species richness (54). Myxomycetes that occur mostly on the bark surface of living trees, shrubs, woody vines, prairie and desert plants fall into five pH groups: low pH (3.5–4.5), mid-range pH (4.6–6.0) and pH (6.1–7.5), high pH values (7.6–10.0), and a broad spectrum of pH (3.5–7.5). When more environmental parameters are better known myxomycetes may one day serve as the basis for evaluating the impact of pollutants on living trees.

1. Department of Biology and Earth Science, University of Central Missouri, Warrensburg, MO 64093, and Botanical Research Institute of Texas, 500 East 4th Street, Fort Worth, TX 76102. U.S.A. Corresponding author: haroldkeller@hotmail.com.

2. Department of Plant Pathology, University of Georgia, Athens, GA 30607-7274. U.S.A.

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Introduction

Myxomycetes often are referred to by mushroom hunters as "slime molds" or "slimes" when collected as plasmodia in the field, but are also known as acellular slime molds, plasmodial slime molds or true slime molds (*Physarum polycephalum*). There are different groups of the so-called "slime molds" that are sometimes confused with each other, for example, the endobiotic and parasitic slime molds (*Plasmodiophora brassicae*) a serious parasite of cabbage, the cellular slime molds (*Dictyostelium discoideum*) also referred to as the "social amoebae," the acrasid cellular slime molds (*Acrasis rosea*), and the protostelid slime molds, a group more recently discovered in the late 1950s (*Protostelium mycophaga*). The latter three groups are microscopic and require laboratory culture for observation. Another unrelated group, the "net slime molds" (*Labyrinthula zosterea*), occurs in estuarine marine habitats associated as leaf parasites of sea grasses and marine algae. The phylogenetic relationships of all of these groups have changed over time as more evidence has accumulated on their life cycles, ultrastructure, and DNA analysis (Martin and Alexopoulos, 1969; Alexopoulos et al., 1996; Keller and Braun, 1999; Spiegel et al., 2004).

Myxomycetes on the Tree of Life

Higher order classification of the Myxomycetes has been controversial when based on different stages in the life cycle. Myxomycetes have been classified as plants in the Kingdom Plantae, Phylum Myxomycophyta, Class Myxomycetes emphasizing the fruiting body stage with walled spores and also in the Kingdom Fungi, Phylum Myxomycota, Class Myxomycetes. The animal-like, motile, amoeboid stages (myxamoebae and plasmodia) and the motile swarm cells (see Fig. 1) were emphasized for including this group in the Kingdom Animalia, Class Mycetozoa, and in the Kingdom Protista, Class Eumycetozoa (Martin and Alexopoulos, 1969; Olive, 1975; Spiegel et al., 2004).

Myxomycetes are currently classified as Myxogastrids in the Super Class Amoebozoa and in the first rank Eumycetozoa (Adl et al., 2005). This follows The International Code of Zoological Nomenclature and the proposed classification of protists by the International Society of Protistologists (Adl et al., 2005). The

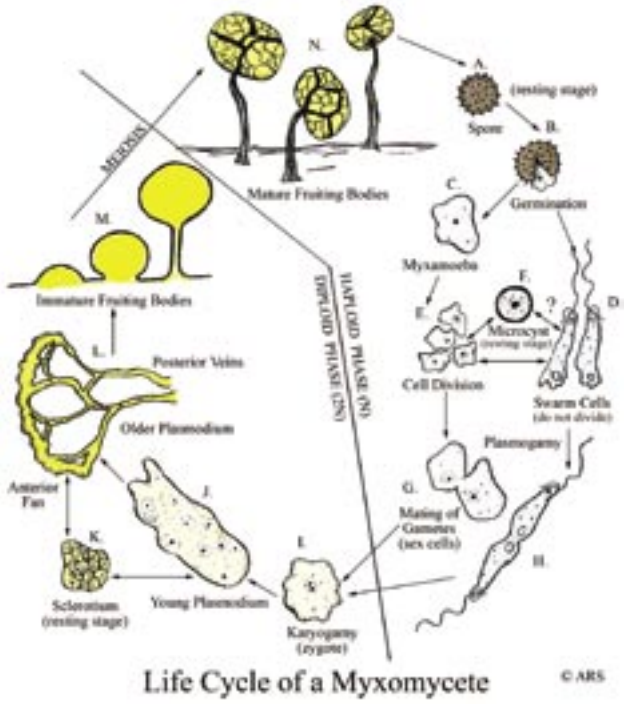

Figure 1. Freehand drawing of Physarum viride *stalked sporangia and different life cycle stages.*

Myxomycetes are no longer recognized as a formal taxon but remains a useful and more commonly recognized term. Despite formal classification as Amoebozoa, Myxomycetes will continue to be studied by mycologists, and classification of species will likely continue to follow The International Code of Botanical Nomenclature due to the practicality of retaining the system and difficulty in transitioning to another nomenclatural system.

The most recent classification scheme of protists supports early observations that endoparasitic, net, and acrasid slime molds are distantly related to the other slime molds, with Peronosporomycetes and Labyrinthulomycetes being placed in Chromalveolata: Stramenopiles, and the Acrasidae assigned to Excavata: Heterolobosea (Adl et al., 2005). Plasmodial, cellular, and protostelid slime molds are thought to be closely related, with current classification assigning them all to Amoebozoa: Eumycetozoa (Adl et al., 2005).

Early phylogenetic analysis (based on Elongation Factor 1-alpha gene sequence) of the Eumycetozoans (Mycetozoans) supported implications that Myxogastrids, Dictyostelids, and Protostelids are a monophyletic group with Protostelids as an ancestral group (Baldauf and Doolittle, 1997). The most recent phylogenetic analyses of the small subunit ribosomal RNA gene (SSU) from many more Eumycetozoan species indicated the Myxogastrids and Dictyostelids are monophyletic clades, but showed Protostelids as a polyphyletic group of seven clades, warranting use of the term protosteloid amoebae to refer to members in these groups (Shadwick et al., 2009). Within the Myxogastrids, deeper phylogenetic analysis based on EF1α and SSU rRNA sequences showed three distinct and well-supported clades, the Echinosteliales, dark-spored orders (Stemonitales and Physarales), and light-spored orders (Liceales and Trichiales), with the Echinosteliales considered the ancestral clade (Fiore-Donno et al., 2005).

Phylogenetic considerations aside, Myxomycetes is still a general term used by myxomycologists. For example, this was the case at The International Congress on Systematics and Ecology of Myxomycetes (ICSEM1; 1993, University College Chester, Chester, England, Great Britain), ICSEM2 (1996, Real Jardín Botánico, CISC, Madrid, Spain), ICSEM3 (1999, National Fungus Collections, Beltsville, Maryland, United States of America), ICSEM4 (2002, National Botanic Garden of Belgium, Meise), ICSEM5 (2005, Universidad Autónoma de Tlaxcala, Tlaxcala, Mexico), ICSEM6 (2008, Nikita Botanic Garden, Yalta, Crimea, Ukraine), and the 7th congress scheduled for 2011 in Recife, Brazil.

The Myxomycete Life Cycle

The myxomycete life cycle (Figure 1, A-N) illustrated by Angela R. Scarborough includes unique features that can be separated into different developmental stages. Fruiting bodies are mostly macroscopic in size and range from about 50 µm to more than 70 cm in diameter with reproductive units represented by dormant, haploid spores 4 to 20 µm in diameter. Spores germinate under moist conditions producing haploid sex cells (gametes) either as myxamoebae that move over the substratum feeding on microorganisms, mostly bacteria, or as unequally biflagellate (whiplash flagellar type) swarm cells that swim in a corkscrew rotation motion in free water, and both of these may mate as sexually genetic compatible gametes. The plasmodial stage that is often brightly colored and conspicuous on ground litter or decaying logs ranges in size from microscopic to several meters in diameter (Keller and Braun, 1999; Everhart and Keller, 2008; Keller et al., 2008). Dormant stages, also called resting or resistant stages, are represented by the spores, microcysts (encysted myxamoebae), and sclerotia (encysted plasmodia). The fruiting body stage, sexual gamete stage, and plasmodial stage can be subjected to experimental variables not possible with many other organisms.

Laboratory Teaching with Myxomycetes

Spore germination experiments are possible during a two-hour laboratory period using *Fuligo septica* aethalia since the spores germinate in most cases within a 30 to 90 minute time period (Braun, 1971). Fresh aethalia (one week old or less) ensure a higher percentage of spore germination but one year old aethalia also will have spores that germinate by the split method forming myxamoebae and eventually swimming swarm cells as long as free water is present (Keller, personal observation).

Hanging drop slides can be used to demonstrate spore germination (Keller and Schoknecht, 1989a). These temporary water mounts are made using a microscope glass slide with a depression well covered with a 22 x 22 mm #1.5 thickness cover slip evenly ringed with petroleum jelly along the four edges. Smear a thin layer of petroleum jelly on the inside palm of the left hand (if you are right-handed) and take the cover slip and scrape the hand surface until the desired thickness accumulates on the edge of the cover slip. This may require trial and error to complete an even amount all along each edge of the cover slip. Place the cover slip with the petroleum jelly-side up on the surface of the microscope slide with one edge overhanging, then put a sterile water droplet in the center of the cover slip. Mix the spores in the water droplet using a dissecting needle (Sundberg and Keller, 1996). This takes time to submerge the spores because some spores have a tendency to float on the surface as a result of surface tension. Carefully lift the cover slip with a needle nose jeweler's forceps and quickly invert it so that the water droplet is hanging downward in the middle. Lower the cover slip on the microscope slide and gently press it to the surface. Practice makes perfect because if the cover slip is tilted the water droplet is displaced and moves to the edge. If too much petroleum jelly is used it interferes with the water droplet. Correctly prepared slides can be placed in plastic Petri dishes to prevent desiccation. The ringing compound must entirely seal the cover slip to the microscope slide or it will dry out before the spores germinate (Keller, personal observation).

Spore dispersal mechanisms can be demonstrated with mature fruiting bodies that have a dehiscent peridium and a powdery spore mass. Elaters characteristic of the genus *Trichia* as defined by Keller and Braun (1999), have free, usually unbranched elastic capillitial threads with surface ornamentation of spiral bands. Other genera such as *Arcyria, Hemitrichia* and *Metatrichia* also have elastic capillitial threads but have more branching and anastomosing. These capillitial threads are hygroscopic and undergo twisting movements when responding to humidity changes in the surrounding environment. This can be simulated in the laboratory by using for example, *Trichia favoginea*, a common myxomycete species found on the underside of decaying logs or pieces of decaying wood in leaf litter. This species can be found throughout the summer, fall, as well as during warmer periods in midwinter. However, all of the above genera have common species found on decaying wood that typically form large colonies of sporangia. Another species that works equally well with hygroscopic action is *Hemitrichia serpula* that forms distinctive plasmodiocarps on decaying logs and wood fragments buried in leaf litter. Fruiting bodies should be placed on a glass microscopic slide with the peridium open or broken open with a dissecting needle and a small droplet of water placed beside the fruiting body or dropped from above. This operation should be done under a dissecting microscope at 20 to 40X magnification. The spore mass acts like a wick absorbing the water. A light source that generates heat should be directed as a pinpoint beam to dry out the fruiting body. The elaters twitch and move, scattering the spores. Students have a greater appreciation for the functional action involved in releasing airborne spores.

Rainfall induced spore dispersal can be demonstrated with species of *Lycogala* fruiting bodies (aethalia) that are mature and have a powdery spore mass. These fruiting bodies look much like puffballs and once were considered such by early taxonomists. One of the best examples is *Lycogala epidendrum* that forms large numbers of aethalia on decaying logs several weeks after rainy periods as the wood gradually dries. Select a single aethalium with a small opening at the top or make a small pore-like hole with a dissecting needle and place the aethalium on a glass microscope slide so the hole in the peridium is centered in an upright position. Use a Pasteur pipette with a rubber bulb to deliver small droplets of water from a height of approximately 4 to 6 cm. When the water drops contact the aethalial surface a small puff of spores simulates what happens in nature. This can be observed with the unaided eye or with a dissecting microscope at 20 to 40 X magnifications. Another easier way is to squeeze the sides of the dried aethalium several times and spores will puff out the opening each time pressure is applied.

The moist chamber culture technique affords the opportunity to study developing myxomycete life cycle stages such as the plasmodium and fruiting body at any time of year. There are a number of publications that describe how to prepare moist chamber cultures for beginners (Braun and Keller, 1977; Keller and Braun, 1999; Keller, 2004; Keller et al., 2004; Keller et al., 2008) and for research professionals (Gilbert and Martin, 1933; Snell and Keller, 2003; Snell et al., 2003; Everhart et al., 2008; Everhart et al., 2009; Kilgore et al., 2009; Scarborough et al., 2009).

Many sources of organic matter can be used for moist chamber cultures: bark from living trees and woody vines (corticolous myxomycetes) (Snell and Keller, 2003), *Juniperus virginiana* being one of the best trees for species richness (Keller et al., 2009); woody parts of cacti and desert succulent plants (succulenticolous myxomycetes) or shrubs (Lado et al., 1999); aerial parts of trees (pine cones) especially species of *Pinus* (for more information on how to access the tree canopy using ropes see Kilgore et al., 2008); legume seed pods in the tree canopy such as *Cercis canadensis* (red bud trees and others). The most frequent collections are made from ground sites on decaying leaves (foliicolous myxomycetes) and decaying logs (lignicolous myxomycetes) (Keller and Braun, 1999). Other special habitats include herbaceous prairie forbs *Asclepias syriaca* (milkweed follicles), *Echinacea* (prairie cone flower) dried composite heads (herbicolous myxomycetes) (Kilgore et al., 2009), on the inflorescences of neotropical herbs (floricolous myxomycetes) (Schnittler and Stephenson, 2002), litter near melting snowbanks (nivicolous myxomycetes)

(Meyer and Bozonnet, 2002), associated with fungi (fungicolous myxomycetes) (Keller et al., 2008), bryophilous myxomycetes that grow and fruit on mosses and liverworts (Stephenson and Studlar, 1985), epiphytes associated with the bark of living trees and woody vines (Everhart et al., 2009), and animal dung (coprophilous myxomycetes) (Krug et al., 2004). One of us (HWK) taught a university course titled "Biology of the Slime Molds" that included each student gathering their own samples for moist chamber cultures. Students shared their culture observations and created a survey list of myxomycete species that usually resulted in some rare or unusual species, including some from their own backyard.

Observation of plasmodia and protoplasmic streaming piques the curiosity of students and beginners of all ages when first encountered as myxomycetes in the field or in moist chamber cultures (Smith and Keller, 2004). The phaneroplasmodium type is the largest sometimes exceeding several meters in the field and covering extensive areas in moist chambers (see Fig. 1L). When the plasmodium becomes visible to the unaided eye it exhibits polarity and directional movement terminating anteriorly in an advancing, fan-shaped, feeding edge and posteriorly in a trailing network of veins. This living mass of protoplasm is not surrounded by a cell wall so it is constantly changing shape as it undergoes shuttle, rhythmic, reversible protoplasmic streaming described in much greater detail by Gray and Alexopoulos (1968, pages 127-149). These observations can be made with lower magnifications of 20 to 40 X using a stereoscopic dissecting microscope.

There are many simple experiments students can do with the plasmodial stage besides the wonder of watching the protoplasmic streaming. Students can use a stopwatch and record the time it takes for anterior movement, pausing, and posterior movement to determine if there is more time spent moving in one direction or the other and if this relates to directional movement across the agar surface or filter paper.

The sclerotium (encysted plasmodium of *Physarum polycephalum*, see Figure 1K) is available as a part of a starter kit complete with sterile oat flakes for food with instructions from Carolina Biological Supply Company. Observation over time will eventually show the network of "plasmodial tracks" on the surface of the agar, filter paper, or on the substratum in nature such as decaying leaves or the bark of living trees that indicates myxomycetes are present. These "plasmodial tracks" are the former living plasmodial veins that have deposited excreted matter along their margins that look like "miniature railroad tracks" with two black lines (edge of vein) and a clear space in between (bottom of the vein). It is interesting to note that an actively feeding plasmodium will not re-cross these tracks, perhaps to avoid re-engulfing harmful substances previously excreted.

One of the properties of the plasmodium, apart from its large size and reversible protoplasmic streaming, is the precise mitotic nuclear division that is synchronous so every nucleus is in the same stage of division: prophase, metaphase, anaphase, or telophase. This was recorded in time-lapse photographic films that show the intranuclear chromosomes moving through these various stages (Koevenig et al., 1961). The transition from plasmodium to immature and mature fruiting bodies with all of the spectacular color changes was compressed from hours into seconds. These three historic films (Slime Molds I, II & III) have been collected on one video (now also on one DVD), running time about 65 minutes. The films were prepared by James Koevenig in 1961 at the University of Iowa under the direction of C. J. Alexopoulos, G. W. Martin and R. T. Porter. These films feature live-action, time-lapse photography, and photomicrography, that teaches about the fascinating world of myxomycetes. A descriptive list of educational resources using myxomycetes is available in the Mycological Society of America (MSA) Newsletter INOCULUM available on line at the MSA website www.msafungi.org (Keller and Everhart, 2006; Part I and Part II).

Myxomycetes in Folklore and Movies

The full scope of fungi and myxomycetes portrayed in folklore literature and the science fiction movie genre is brought to life in the detailed and elegant prose of Rose (2009). This paper should be consulted for the horror and scary tales associated with myxomycetes that has created a special fascination with the general public. The plot of the movie script has some form of living matter "the blob" or "spores" that arrives from outer space and feed on or terrorize humans. One example that made local and national newspaper headlines was "the blob" that was found in the backyard of a homeowner in Garland, Texas, a suburb of Dallas. These yellow blobs (a slime mold plasmodium) were present for a three-week period and the fruiting bodies were finally identified as *Fuligo septica* but not after concern that a lawn disease or alien source had invaded the site (Alexopoulos et al., 1996, page 779; Everhart, 2006; Keller and Braun, 1999; Nieves-Rivera, 2000; Nieves-Rivera, 2001).

Interest in Myxomycetes as Plant Pathogens

Are any myxomycete species plant pathogens (see Nieves-Rivera, 2000)? Certain myxomycete species are often found on decaying leaf litter or grass used as mulching in gardens. Under ideal conditions of moisture and temperature numerous fruiting bodies of *Diachea leucopodia*, *Fuligo septica*, *Mucilago crustacea*, *Physarum cinereum*, and species of *Stemonitis* also may cover living garden plants or plants in greenhouses (Keller, personal observation). In these cases the plasmodium migrates up the stems or on the leaf surface forming fruiting bodies that would make edible plants unappetizing and therefore inedible. It is noteworthy that all of these species also occur on decaying ground litter of leaves and twigs. However, since there is no penetration of plant tissues and

no symptoms produced, myxomycetes should not be considered plant pathogens.

Another example is related in the following account based on fruiting body formation of *Physarum cinereum* on living Saint Augustine lawn grass at Arlington, Texas (Keller and Braun, 1999). ". . . the fruiting bodies scattered over 0.25 sq m or more. Sometimes, these fruitings will form a ring and, like some mushrooms, these are called 'fairy rings.' Immature stages of fruiting bodies still soft and moist on living lawn grass are blackish. At this time, the green grass has a blackish tinge and many urban home owners express concern that their grass is sick. In a few hours, as the fruiting bodies dry out, they have a whitish color due to the formation and precipitation of calcium carbonate in the peridium. *Physarum cinereum* has been implicated in killing grass, but little damage is done by the fruiting bodies forming on the blades of grass; usually mowing the lawn or hosing the area with water will remove the cause for concern. Resting stages apparently survive in the soil because fruiting bodies appear year after year in exactly the same spot on lawn grass (Harold W. Keller, unpublished observations)." A popular account of slime molds on lawns highlights this topic for a general readership (Everhart, 2006).

Another species, *Physarum bogoriense,* also occurs on living blades of Saint Augustine grass in lawns known from sites at Arlington, Texas. The development of the fruiting bodies in different stages of maturation was observed and highlighted by the preformed reticulate lines of dehiscence that eventually form a distinctive three-layered peridium with reflexed lobes (Keller and Braun, 1999). This species usually is found on decaying leaf litter in tropical and subtropical areas (Whitney and Keller, 1982) and less frequently from more temperate habitats.

Despite the presence of human parasites within the same super class as the myxomycetes and evidence for flagellate protista as phytopathogens, these groups are distantly related and there is no evidence that myxomycetes are parasitic or phytopathogenic. In fact, out of the more than 200,000 named species within the Super Class Amoebozoa, only about 10,000 (5%) are known parasites, with even fewer of those representing parasites of importance (Cox, 2002). Amoebae are an under-studied group of organisms in part due to their paucity of disease-causing species that would otherwise increase their importance for human welfare and economic impact.

Fruiting Bodies

Morphological characteristics of myxomycete fruiting bodies are used for the identification of the myxomycete genera and species. Some myxomycetes can be picture-keyed and identified using the unaided eye. The majority of approximately 900 species require either preferably a 20X magnification hand lens, or a microscope slide mount preparation and the use of a compound microscope at 1000X magnification (oil immersion lens) to determine ornamentation of internal structures such as the peridium, capillitial threads, and spores.

Myxomycete fruiting bodies: does size really matter? The colorful plasmodium attracts collectors and photographers resulting in images submitted to photographic contests that often win the top prizes at the North American Mycological Association national meetings as well as state and local mycological society meetings. This coupled with the fact that mature fruiting bodies can be collected and preserved in small collecting boxes facilitate the study of myxomycetes by a broad spectrum of age groups from children to adults (Keller and Braun, 1999).

Myxomycetes Exhibit Ecological Survival Strategies

Myxomycetes exhibit examples of biological principles in the strategy behind the evolution of size, numbers of fruiting bodies, stalked habit, spore size, shape, and ornamentation. There is a size transition from the genus *Echinostelium* with tiny stalked sporangia and fewer spores to the large aethalia of *Fuligo septica* that may be more than 70 cm across and with billions of spores. Tiny sporangia and protoplasmodia occur more frequently on the bark surface of living trees and woody vines (corticolous myxomycetes) and more rarely on ground sites. The evolutionary strategy behind the most abundant corticolous myxomycetes is the formation of the microscopic plasmodial type (protoplasmodium) with the smallest surface to volume ratio, rapid sporulation in two to four days producing single tiny, stalked sporangia, and the release of spores via an evanescent peridium. Corticolous myxomycetes apparently cannot compete well for space with other organisms on ground sites. Their life cycle strategy exhibits the r-selection scheme on the bark of living trees and woody vines that is unpredictable or short-lived, with a cycle of unfavorable (extreme hot, dry and cold periods) much like the desert ephemeral plants that take advantage of brief periods of rain and germinate, grow, flower and set seed in a matter of weeks. In the case of myxomycetes, sporangia may appear in moist chamber cultures in 24 hours and should be examined shortly after adding water or these tiny sporangia will be overlooked. In contrast typical myxomycete species that occur on decaying wood or leaves (ground species) follow the K-selected populations that have more favorable conditions (longer, wet, optimal moderate temperatures) and typically appear much later in moist chamber cultures up to several weeks or a month (Everhart and Keller, 2008).

Stalked versus sessile fruiting body numbers were noted in a tree canopy study of corticolous myxomycetes on living tree species and woody vines by Everhart et al. (2008). A total of 46 myxomycete species included 28 species with stalked sporangia (60.9%), 13 species of sessile sporangia (28.3%), and aethalia, pseudoaethalia, and plasmodiocarps (10.8%) represented by five species. The corticolous myxomycete genus *Echinostelium* with

the smallest sporangia most recently was recognized as having all 19 species stalked and likewise the genus *Macbrideola* with 14 species (Lado, 2001). In his catalog of corticolous myxomycetes, Mitchell (2004) listed 275 species and of these 163 (59%) developed stalked fruiting bodies. The most recent myxomycete world monograph by Martin and Alexopoulos (1969) described a total of 414 species mostly from ground sites. The species descriptions in some cases span the stalked habit to sessile sporangia and plasmodiocarps and others that include the sessile aethalioid and pseudoaethalioid fruiting body types that made a subjective evaluation of each species as being primarily stalked or sessile an estimate. The percentage of stalked sporangia based on 279 species (67.4%) compares closely to previous estimates.

The majority of myxomycete spore sizes falls within the 8.0 to 12.0 µm range, the smallest approximately 4.0 µm (*Stemonitis smithii*) (Martin and Alexopolous, 1969) and the largest (20 µm) *Fuligo megaspora* (Keller and Schoknecht, 1989b) that has unique spore ornamentation (an episporic reticulum with a serrated edge). Why do the spore size, shape, and ornamentation matter? The majority of myxomycete spores are spherical and a few are elliptical or ovoid with one exception, *Badhamia ovispora*, that was incorrectly described as having ovate spores, but instead, has hotdog shaped spores with unique plaque-like raised areas (Keller et al., 1975). There are only a few species of *Licea* (for example *L. parasitica*) and *Badhamia apiculospora* that have smooth-walled spores (Raub et al., 1979); less than 10 species out of the 867 listed (and now more likely near an estimated 900 species) in Lado (2001). This compares with the far fewer approximately 100 species of acellular slime molds that have smooth walled, elongate/oblong spores that occur mostly on ground sites in soil, forest leaf/needle litter, and on dung. This would suggest selective evolutionary pressure in the myxomycetes toward spore roundness, ornamentation, and size to maximize long-range airborne spore dispersal. In addition, the absence of square, triangulate, appendages, or tetraradiate spores, the latter adaption found in aquatic fungi, also argues for dry airborne spore dispersal in the myxomycetes.

Primary and Secondary Myxomycete Spore Dispersal Agents

Most myxomycete fruiting bodies have windborne spores as the primary agent of dispersal because of the powdery spore mass, evanescent or fragile peridium that breaks apart at maturity, and relatively small spherical spores with some kind of ornamentation. Secondary agents of spore dispersal may involve different invertebrates such as insects either by ingestion (Keller and Smith, 1978) or by specialized structures (Stephenson and Stempen, 1994). Arthropod assemblages that are spore dispersal agents include mites, flies, and beetles. The beetles are either accidental or obligately found within the fruiting bodies and carry the spores to other landing sites. Many new species of slime mold beetles have been described in the genera *Anisotoma* and *Agathidium* and appear to feed as specialists on slime mold plasmodia and fruiting bodies (Wheeler, 1984a; Wheeler, 1984b). Other interactions with slugs (Keller and Snell, 2002) and nematodes (Kilgore and Keller, 2008) involved feeding activities associated with myxomycetes.

Biological Principle of Convergent Evolution

There is selective evolutionary pressure in microorganisms for a stalked habit and propagating units that are raised off the substratum so they are airborne. For example, the myxobacteria (gliding bacteria) through an aggregative phase form a stalk and complex (fairly large) bodies in *Chondromyces* and *Stigmatella* that are colorful and can be seen in some cases with the unaided eye or at low magnification. The stalked bodies have walled sporangioles and myxospores that are often seen along with myxomycetes in moist chamber cultures with bark from living trees. The myxobacteria are classified in the Kingdom Bacteria and are prokaryotes compared to myxomycetes that are eukaryotes and included in the Kingdom Protista (Reichenbach, 1993).

Other examples include a sorogenic ciliate, *Sorogena stoianovitchae*, which forms an aggregation of amoeboid cells. This ciliate feeds voraciously as a ciliate on a species of *Colpoda*, and when this food organism eventually is depleted in numbers, the ciliate develops a stalk with a sorus of cysts at the apex (Olive, 1975; Bradbury and Olive, 1980).

The dictyostelids (the cellular slime molds), such as *Dictyostelium discoideum*, have a free living amoeboid phase that eventually aggregates into a migrating slug then forms a stalked sorocarp with a spore mass at the tip (Bonner, 2009). The microscopic protosteloids (the protostelids of Olive, 1975) have myxamoebae that form a thin delicate stalk with a single spore at the tip. The myxomycetes have myxamoebae that develop into an aggregative mass of protoplasm, the plasmodial phase (Fig. 1, L), and eventually form the stalked fruiting body (sporangium Fig. 1, N).

There is a progression of increase in the size of stalks from the ciliates, protostelids, dictyostelids, and myxobacteria, all microscopic except when forming masses of fruiting bodies that still require a 20X hand lens and a sharply trained eye (Spiegel et al., 2004). The myxomycetes have stalked fruiting bodies and plasmodia that span the spectrum of size from microscopic to covering an area of more than a meter. These taxa may be separated by classification at the kingdom, phylum, and class levels and represent examples of convergent evolution that convert amoeboid cells into some kind of airborne structure with spores.

Myxomycetes and Mating Types

Tester myxamoebal clones using heterothallic (+) and (-) sexual strains carrying only one sexual mating type locus, represent sex-

ual gametes, the sperm and egg of mammals. The myxomycetes are unique in all living organisms since these sexual gametes can be cultured in perpetuity becoming potentially immortal and can build up populations of gametes through growth and division. Myxamoebae can be transferred from culture to culture as sexual gametes, and where this is possible, resulted in evidence that the genetic mating type system operated as a single locus-multiple alleles. Collins and his students put *Didymium iridis* and myxomycetes on the genetic map as the system of choice to study mating types, apogamy, apomicts, and plasmodial incompatibility (Collins, 1979; Collins, 1981). His pioneering discovery of homothallism and heterothallism in *Physarum* and *Didymium* still stands today as one of great mileposts of scientific advancement. Collins was a world-famous African-American botanist/fungal/slime mold geneticist with continual financial support from the National Science Foundation over many years; unfortunately, his research on reproductive biology, genetics, and speciation was cut short by his death in 1989 at the age of 58 (Taylor and Silliker, 1993).

Myxomycetes and Aging Research

Experiments designed to determine life span and senescence in plasmodia of *Didymium iridis* and *Physarum cinereum* showed that aging/longevity were under genetic nuclear control and not cytoplasmic factors (Clark, 1984; Clark and Lott, 1989). This research was financially supported by the National Institute on Aging.

Physarum polycephalum as a Model Organism in Cancer Research

The research of Harold P. Rusch and his associates at the McArdle Laboratory for Cancer Research, University of Wisconsin, with financial grant support from the National Cancer Institute, used a chemically defined medium to culture the *P. polycephalum* plasmodium axenically under controlled laboratory conditions (Cummins and Rusch, 1968; Rusch, 1970). This giant amoeba functions as a single cell but chemical events associated with growth, mitosis, differentiation, cytokinesis, and karyokinesis are separate and distinct events. The mitotic synchronous cycle could be the result of a biological clock in the nucleus or caused by a cytoplasmic factor. This question is important and has direct relevance when compared to unregulated cell divisions that have lost cellular internal control in the formation of cancerous tumor cells. Experiments showed that mitotic synchrony is not triggered inside the nucleus but the stimulator accumulates in the cytoplasm and is transferred to the nucleus shortly before mitosis (Cummins and Rusch, 1968; Rusch, 1970).

The most remarkable example of myxomycetes enabling cancer treatment is with a non-toxic, non-immunogenic, and biodegradable nanoconjugate drug delivery system called Polycefin. Polycefin is synthesized from purified Poly(β-L-malic acid) (by *P. polycephalum*) and is subsequently modified for directed delivery of morpholino antisense oligonucleotides (gene silencing therapy), antibodies, and anti-tumor drugs to certain tumor cells. Preliminary testing showed that fluorescently labeled Polycefin can be injected in to the tail vein of a mouse and accumulates within breast and brain tumor cells (Ljubimova et al., 2008).

Myxomycete Research in Outer Space

The plasmodium of *P. polycephalum* has been used in the American, German, and Russian space programs. Experiments on board the Russian biosatellite Kosmos-1129 in 1979 demonstrated the migration of the plasmodium and protoplasmic streaming was maintained and survived under microgravity conditions (Tairbekov et al., 1984). A joint mission in 1986 between the German Spacelab Mission D-1 and the United States National Aeronautics and Space Administration (NASA) reported using the plasmodium of *P. polycephalum* as a model system in biorack experiments to study contraction behavior and graviresponse of a single mass of living protoplasm that possesses no receptors specialized to a gravity response. The results showed a clear sensitivity to gravity and phototaxes. The weightlessness experiments in space confirmed the validity of zero-gravity simulation on the fast-rotating clinosat (Block et al., 1986; Block et al., 1994).

Myxomycete Spore Aeroallergens

Myxomycete spores are mostly windborne. Photographic arrays taken from mold spore air samples on microscope slides showed two species of myxomycetes that were identified with confidence because of size and ornamentation. *Fuligo septica* had spinulose spores 6.0 to 8.0 µm in diameter and the spiny reticulate spores of *Stemonitis fusca* were distinctive (Keller, personal observation). There are cases where *F. septica* aethalia when handled or broken open released spore plumes that resulted in sneezing episodes (Keller, personal observation). In addition, both of these species are common, cosmopolitan, and abundant, producing significant numbers of spores during rainy periods.

A human subject population of 250 individuals with rhinitis and/or asthma was part of a clinical study where intradermal testing was performed using a pure spore extract of *F. septica* (Rockwell et al., 1989). Reactivity in this population was 40 %. A subset of positive and negative subjects was given a radioallergosorbent test and this RAST protocol is a blood test used to determine what substances are allergenic. There was a 95 % concordance between the RAST and skin testing. This study concluded that *F. septica* was an important aeroallergen and should be used in the diagnosis and treatment of atopic patients (Rockwell et al., 1989). Individuals hypersensitive to mold spores should use face masks to avoid contact with slime mold spores produced by common aethalioid taxa such as *Enteridium*, *Fuligo*, and *Lycogala* and sporangia produced in massive numbers such as certain taxa of *Physarum* and *Stemonitis*.

Table 1. Novel chemical compounds from the fruiting body and plasmodium of myxomycetes

Myxomycete species	Life cycle stage	Novel compound	Important function	Literature Citation
Arcyria denudata (L.) Wettst.	fruiting body	arcyriaflavin A and B	moderately antibiotic	2
		arcyroxocin B	cytotoxic to Jurkat cells	2, 20
		new bisindole sulfate	unknown	20
A. cinerea (Bull.) Pers.	fruiting body	cinereapyrrole A and B	unknown	18
A. ferruginea Saut.	fruiting body	arcyriaflavin C	inhibits Wnt cell signal, antibiotic	2, 22
		arcyriarubin C	inhibits Wnt cell signal, antibiotic	2, 20, 22
A. nutans (Bull.) Grev.*	fruiting body	arcyriacyanin A	cytotoxic to Jurkat cells, antibiotic	2, 15
		arcyroxocin A	unknown	2
Cribraria cancellata (Batsch) Nann.-Bremek.	fruiting body	cribrarione B	unknown	10
C. intricata Schrad	fruiting body	lindbladione	naphtoquinone pigment	13
C. meylanii Brândză	fruiting body	cribrarione C	unknown	23
C. purpurea Schrad.	fruiting body	cribrarione A	antimicrobial against *Bacillus subtilis*	14
Dictydiaethalium plumbeum (Schumach.) Rostaf. ex Lister	fruiting body	arcyriaflavin D	moderately antibiotic	2
Didymium bahiense Gottsb.	plasmodium	makaluvamine	cytotoxic topoisomerase II inhibitor	8
D. bahiense var. bahiense Gottsb.	plasmodium	bahiensol	antimicrobial against *Bacillus subtilis*	12
D. squamulosum (Alb. & Schwein.) Fr.	fruiting body	clionasterol	unknown	7
Fuligo septica (L.) F.H. Wigg.*	fruiting body	cycloanthranilylproline	unknown	16
F. septica f. flava (Pers.) Y. Yamam.	fruiting body	fuligoic acid	yellow pigment	23
Lindbladia tubulina Fr.	fruiting body	lindbladione	naphtoquinone pigment	9, 13
Lycogala epidendrum (J.C. Buxb. ex. L.) Fr.	fruiting body	2 new bisindole alkaloids	cytotoxic to HeLa, Jukat, and vincristine resistant cells; one inhibited protein tyrosine kinase activity	17
		arcyriaflavin A and B	moderately antibiotic	2, 3, 17
		arcyriarubin A	moderately antibiotic	2, 3
		lycogalic acid A and B	unknown	3, 5
		lycogarides A-G	unknown	5
		lycogarubins A-C	C showed anti-HSV-I virus activity	4
		staurosporinone	derivatives are clinical candidates	3, 21
Physarum melleum (Berk. & Broome) Massee	plasmodium	melleumin A and B	unknown	16, 19
P. polycephalum Schwein	plasmodium	physarochrome A	pigment (photoreceptor)	1
		polycephalin B and C	pigment (photoreceptor)	6
P. rigidum (G. Lister) G. Lister ex Lister	plasmodium	physarigins A-C	pigment	11
Trichia favoginea var. persimilis (P. Karst.) Y. Yamam.	fruiting body	kehokorins A-C	cytotoxic to human epithelial carcinoma cells	20
Tubifera casparyi (Rostaf.) T. Macbr.	fruiting body	arcyriaflavins B and C	moderately antibiotic	2, 21
T. dimorphotheca Nann.-Bremek. & Loer.	fruiting body	tubiferal A and B	A restores vincristine drug sensitivity to human epidermoid carcinoma cells	15

Literature citations: 1. Steffan et al. 1987, 2. Steglich 1989, 3. Fröde et al. 1994, 4. Hashimoto et al. 1994, 5. Buchanan et al. 1996, 6. Nowak and Steffan 1998, 7. Ishibashi et al. 1999, 8. Ishibashi et al. 2001, 9. Ishikawa et al. 2002, 10. Iwata et al. 2003, 11. Misono et al. 2003a, 12. Misono et al. 2003b, 13. Misono et al. 2003c, 14. Naoe et al. 2003, 15. Kamata et al. 2004, 16. Nakatani et al. 2004, 17. Hosoya et al. 2005, 18. Kamata et al. 2005, 19. Nakatani et al. 2005, 20. Kamata et al. 2006, 21. Sánchez et al. 2006, 22. Kaniwa et al. 2007, 23. Shintani et al. 2009.

*These names represent the currently accepted nomenclature where synonyms were reported in the cited literature

Isolation of Novel Compounds with Biological Activity

There are more than 100 secondary metabolites that have been isolated from myxomycetes that can be categorized as lipids, fatty acid amides and derivatives, alkaloids, amino acids and peptides, naphthoquinone pigments, aromatic compounds, carbohydrate compounds, and terpenoid compounds (Dembitsky et al., 2005). The detailed description of these compounds, including their structure and biological activity, was in the review by Dembitsky et al. (2005). The compounds selected here represent novel compounds first described from extracts of myxomycetes or are compounds with an important biological activity (Table 1).

Early studies of myxomycetes for the production of compounds with biological activity often focused on isolating and testing crude extract of the plasmodium or fruiting bodies. In certain cases, the crude extract was obtained from a plasmodium cultured with *Escherichia coli,* and showed antibiotic activity, however, there was no way to determine if the biologically active compound was from the myxomycete compounds or the bacterium. Indeed, many crude extracts of myxomycete fruiting bodies and plasmodia have shown antimicrobial properties but did not yield compounds with antimicrobial activity. For example, crude extract of *Physarum melleum* exhibited antimicrobial properties against *Bacillus subtilis* but the extracts, melleumin A and B, did not show antimicrobial activity against *B. subtilis,* and therefore, it is thought that the compounds found in the crude extract that show antimicrobial activity are unstable yellow pigments in the plasmodium (Nakatani et al., 2005). In the case of field-collected fruiting bodies of *Cribraria cancellata,* the crude extract also showed antimicrobial activity against *Bacillus subtilis*, but the secondary compound, cribrarione B, did not exhibit antimicrobial activity (Iwata et al., 2003).

Among the 22 myxomycete species that were examined for novel compounds none included species in the Echinosteliales (microscopic protoplasmodia) or Stemonitales (aphanoplasmodia). Many papers describing the extraction of compounds from myxomycetes involve tedious chemical procedures that also include steps for the synthesis of the compound of interest (Steglich, 1989). Sufficient quantities are necessary before physiological investigations can begin, which is why an important function of the compound may yet be unknown or not thoroughly investigated for antimicrobial, cell-directed, or inhibitory activities. Most secondary metabolites isolated from myxomycete fruiting bodies and plasmodia are directly responsible for pigmentation (Steglich, 1989), and there are a number of compounds that have shown some promising biological activities.

Arcyriacyanin A, isolated from *Arcyria nutans* (Steglich, 1989), has shown inhibitory activity against a number of human cancer cell lines, inhibits protein kinase C, and inhibits protein tyrosine kinase (Hibino and Choshi, 2002). It is important to know if the compound expresses inhibitory activity against protein kinases because these proteins are critical in regulation of cell growth and differentiation that is encoded by many oncogenes. *In vitro* assays were conducted on 39 human cancer cell lines, including those taken from tissues in the lung, stomach, colon, ovary, central nervous system, breast, renal, skin, and prostate (Murase et al., 2000). This revealed that the effective dose was high but the method of action of this compound may be worth further investigation. It is possible that arcyriacyanin A or precursors discovered during the synthesis process could be used to inhibit cancer cell growth.

Arcyriaflavin A, isolated from *Arcyria denudata* and *Lycogala epidendrum* (Steglich, 1989; Fröde et al., 1994; Hosoya et al., 2005), has been shown to have moderate antibiotic activity against fungi and bacteria and is able to inhibit signals in the Wnt cell signaling pathway. Biosynthesis of compounds of particular interest, such as arcyriaflavin A, has also been achieved through genetic modification of a biosynthetic pathway (rebeccamycin) in an actinomycete (*Streptomyces albus*). In this case, simple modification to induce co-expression of rebaccamycin pre-cursors resulted in the accumulation of arcyriaflavin A, which has known anti-viral properties and was shown to have potential as a cancer cell growth regulator (Sánchez et al., 2005).

Secondary metabolites isolated from myxomycetes that have shown potential for the development of drugs for clinical trials are mostly from a group of compounds called indolocarbazoles. Indolocarbazoles include the arcyriarubins, arcyriaflavins, arcyroxepins, arcyriacyanins, lycogalic acid, lycogarubins. Staurosporine is a compound originally isolated from an actinomycete, *Nocardiopsis* sp. (Kase et al., 1986) and also from *Lycogala epidendrum* (Fröde et al., 1994). Phase II and III clinical trials of a semi-synthetic Staurosporine compound have been tested and show promise as a drug to slow the neuronal degenerative effects of Parkinson's disease (Butler, 2005).

Slime Molds, Intelligence, and Biological Robots

The plasmodium of the slime mold *P. polycephalum* was placed in a labyrinth that was represented by a maze of pathways on the surface of an agar gel Petri dish. Oat flakes were placed at two exit points as a potential food source. The plates without oat flakes at the two exit points resulted in the plasmodium spreading throughout each pathway of the maze. The maze with the oat flakes at the two exit points resulted in the plasmodium migrating through the shortest possible route and minimum possible length solution suggesting that the slime mold could process external stimuli (Nakagaki et al., 2000). The researchers concluded that the slime mold exhibited a kind of "primitive intelligence" and resulted in the press portraying this response as "primitive brainpower."

The first amorphous biological robot (non-silicon plasmobot), using the plasmodium of *P. polycephalum* as the motive

source, has been designed to operate the computer circuitry that will directionally move light-weight floating objects on water and perform concurrent and parallel computations (Adamatzky and Jones, 2008; Adamatzky, 2009). The plasmodium was grown as a six-pointed star on top of a six-legged robot attached to a circuit and connected remotely via a computer to the hexapod bot. A light source was shone on one of the points attached to the circuit so that the protoplasmic streaming and movements either toward or away from a light source controlled the robot. This team of scientists at the University of the West of England received a Leverhulme Trust grant to develop the first fully biological amorphous massively parallel robot.

Myxomycetes as a Human Food Source

The yellow plasmodium of *Fuligo septica* has been reported by Lopez et al. (1982) as eaten by the Indians from the area of Cofre de Perote in the state of Veracruz, Mexico, where it is referred to by the popular name of "caca de luna" or translated into English "excrement of the moon." The scrambled-egg-like stage is fried with onions and peppers much like scrambled eggs and eaten on a tortilla. Almost every tree and shrub with bark mulching had large plasmodial masses of *F. septica* during a rainy period with high temperatures that occurred during the Mycological Society of America annual meeting, July 17–21, 2004, at the University of North Carolina-Asheville campus (Keller, personal observation). This will serve as an example that sufficient quantity of immature plasmodia could be collected in a localized area given optimal environmental conditions. Another myxomycete, *Reticularia lycoperdon,* was described as edible in the young, immature, white fruiting body stage (aethalium) from the states of Veracruz and Tlaxcala, Mexico (Villarreal, 1983). People in Veracruz fry it like eggs, and when eaten it has a pleasant nutty taste.

Species Biodiversity Rule Broken by the Myxomycetes

Species richness increases with warmer temperatures in the tropics for insects, birds, mammals, and flowering plants. Insects especially are diverse and generally much larger in size in the hotter tropics than in cooler climates. Flowering plants are the classical example of higher species diversity in the hotter tropics and decrease in species numbers as geographical movement goes toward the North and South Poles (colder temperatures). The cellular slime molds also show a similar trend of higher species diversity in the tropics and lower at higher altitudes on equatorial mountains (Bonner, 2009). This biodiversity rule applies then to both the macro and microbiological world and is mentioned in biodiversity discussions in many general biology textbooks. However, the myxomycetes are an exception to this rule. The tropical rainforest has the lowest myxomycete species diversity and this is well documented for ground site species (Farr, 1976).

More recently exploration of more tropical areas has resulted in the discovery of a new microhabitat with an assemblage of myxomycetes associated with the inflorescences of neotropical herbs that form the understory plants (Schnittler and Stephenson, 2002). There were 31 different myxomycete taxa on the decaying floral parts enclosed by the living bracts primarily on the species of *Heliconia* and *Costus*. Some of these myxomycete species are also known from ground sites but this raises the possibility that species richness may increase when higher parts of the tree canopy are explored in the tropics. Nevertheless, based on current myxomycete species lists from temperate regions, especially the mixed conifer/hardwood deciduous forests in the U.S.A. (for example, the Great Smoky Mountains National Park), species diversity is much higher there than in the tropics or polar regions of the world (Arctic and Antarctic) (Snell and Keller, 2003; Snell et al., 2003; Keller, 2004).

There are a number of explanations for this unusual geographic distribution in the tropical rain forests: so much rainfall that the plasmodia rarely form fruiting bodies and when they do they get washed away quickly; there is much less leaf litter as found in the temperate deciduous forest where leaves and twigs make up the forest litter; the storied, closed tree canopy reduces air currents so that spores cannot be distributed widely throughout the ecosystem; myxomycetes may not be able to compete with other microorganisms in the soil or on ground sites; or there may be other unknown factors yet to be discovered. This is a biological puzzle that merits more research.

Environmental Importance and Future Ecological Potential

Myxomycetes (swarm cells, myxamoebae, and plasmodia) are phagotrophic bacteriovores and fungivores. The feeding behavior of myxamoebae/swarm cells indicates they will ingest only spores within a certain size range but there does not seem to be any specialization of the myxomycete species to appropriately sized spores (Gilbert, 1928; Olive, 1975). For this reason, it is thought that soil-dwelling myxamoebae and swarm cells play an important role as secondary saprotrophs (Adl and Gupta, 2006).

Some fungivorous secondary saprotrophs, such as gymnamoebae, *Leptomyxa* and the Vampyrellidae (Rhizaria: Cercozoa: Filosea), and the colpodid Grossglockneriididae, may be useful as biocontrol agents (Adl, 2003). These protozoa could be useful in suppressive soils by reducing the number of spores of the soil dwelling plant pathogens, such as *Rhizoctonia solani*, however it is less likely that myxomycetes would be useful as biocontrol agents because they lack specificity in their food source and are not able to penetrate hyphae.

The massive, cushion-shaped aethalium of *Fuligo septica* has billions of spores inside of a thick calcium carbonate crust and also has a large yellow plasmodium that may serve as an experimen-

tal model to study the uptake and concentration of heavy metals such as the hyper-accumulation of zinc (Stijve and Andrey, 1999; Zhulidov et al., 2002). *Fuligo septica* accumulates zinc (395-3,600 mg/kg based on dry matter) compared to 10-160 mg/kg in *Vaccinium* (blueberry leaves). In addition, in lesser but still significant amounts, barium, cadmium, iron, manganese, and strontium were found in *F. septica* in amounts much greater than in macromycetes and micromycetes (Stijve and Andrey, 1999). The biochemical detoxification mechanism of highly toxic levels of zinc in *F. septica* and the cloning of the genes involved could be used in plants with greater biomass for bioremediation of polluted soils.

Myxomycetes that occur mostly on the bark surface of living trees, shrubs, woody vines, prairie and desert plants fall into four pH groups: species associated with a lower pH (3.5–4.5) such as many conifer trees; deciduous trees and woody vines in the mid-range pH (4.6–6.0) that represents the majority of myxomycetes; another group of myxomycetes associated with higher pH (6.1–7.5) for certain trees in temperate areas; and higher pH values (7.6–10.0) for prairie forbs, desert, and semi-arid vegetation; a lower number of species that have a broad spectrum of pH (3.5 to 7.5) (Everhart et al., 2008; Everhart et al., 2009; Keller et al., 2009; Kilgore et al., 2009; Scarborough et al., 2009; Snell and Keller, 2003). These studies were conducted mostly in the Great Smoky Mountains National Park (GSMNP), Daniel Boone National Forest in Kentucky, The Land Institute near Salina, Kansas, and Pertle Springs, Warrensburg, Missouri.

Acid rain, bark pH of living trees, and a combination of aerial pollutants produced by burning fossil fuels (nitrogen oxides and sulfur dioxides), contribute to significant tree injury and death especially at high elevation sites in GSMNP. The living tree species *Abies fraseri* (mean pH 4.07) and *Picea rubens* (mean pH 3.65) located at high elevation sites in GSMNP near the Clingman's Dome area of Tennessee were sampled from the tree canopy (Scarborough et al., 2009) using the Doubled Rope Climbing Method (Kilgore et al., 2009). *Abies fraseri* canopies did not have plasmodia nor myxomycete fruiting bodies, and *P. rubens* had ten species, the lowest species richness when compared to angiosperm trees (Scarborough et al., 2009). Bark from living tree canopies of *Juniperus virginiana* at low elevations in GSMNP (Scarborough et al., 2009) and at locations throughout the southeastern and midwestern USA (Keller et al., 2009) has the highest species richness of myxomycetes (54) of any tree species studied to date.

It is possible that a Biotic Pollution Index could be developed using myxomycete species assemblages and species richness associated with bark pH values taken from different living tree species. More data is needed from many more individual trees that include precise data on tree age based on tree cores, diameter at breast height, and total vertical height. Furthermore, the myxamobae and plasmodia feed on bacteria, yeasts, and other microorganisms that may also have an optimal range of pH requirements. When all of these environmental parameters are better known myxomycetes may one day serve as the basis for evaluating the impact of pollutants on living trees.

Concluding Remarks

Much of the myxomycete research completed in the last 10 years related to natural products was surveyed in the published literature. Thus, this has resulted in a significant increase of natural products isolated from fruiting bodies and plasmodia that resulted in a table where some of this information is organized in one place. However, follow up clinical studies involving human trials must be implemented before any practical use will be possible. Many more topical headings could have been added but constraints of time and space placed certain limits. A large part of this information was selected to update research that in part will increase the significance of the myxomycetes as more than just biological curiosities. The financial support of national granting agencies was added to show that myxomycetes merit recognition in the scientific community as organisms of special significance that can answer basic biological questions. You have to see it to believe it that the seventh grade life science students at Warrensburg Middle School in observing moist chamber cultures expressed so much excitement when observing plasmodia and the formation of fruiting bodies with words such as "awesome" or "cool" that were heard over and over again. In the end, the passion and excitement (HWK) in pursuing the study of myxomycetes for more than 45 years since I collected my first myxomycete specimen as a graduate student at the University of Kansas near Lawrence was because of the aesthetic beauty of these "biological jewels of nature." The majesty and grandeur of the redwood sequoia trees that have been on planet earth for several thousands of years are truly a marvel of nature but the transformation of the myxomycete plasmodium to fruiting body stage in 24 hours with spectacular color changes or the iridescent spectrum of rainbow colors exhibited by the sporangial wall of the recently described *Diachea arboricola* H. Keller & M. Skrabal give special meaning to the words "SMALL IS BEAUTIFUL."

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