INVITED RESEARCH PAPER

Tree Canopy Biodiversity in Temperate Forests: Exploring Islands in the Sky

Harold W. Keller[1,2], Sydney E. Everhart[3], Melissa Skrabal[4], Courtney M. Kilgore[1]

[1]Department of Biology and Earth Science, University of Central Missouri, Warrensburg, Missouri 64093, U.S.A. [2]Botanical Research Institute of Texas, 500 East Fourth Street, Fort Worth, Texas 76102, U.S.A. [3]Department of Plant Pathology, University of Georgia, Athens, Georgia 30602, U.S.A. [4]St. John's Pain Management Center, 2135 South Fremont, Springfield, Missouri 65804, U.S.A.

Key words: All Taxa Biodiversity Inventory, corticolous myxomycetes, cryptogams, Daniel Boone National Forest, Discover Life in America, Great Smoky Mountains National Park, Pertle Springs, Missouri, species biodiversity, tree canopy doubled rope climbing method

"Trees are wild perennial plants that give us beautiful forest landscapes and challenges to explore the treetops for unknown species biodiversity". *Anonymous*

The early years: a flatlander from Kansas

My formative early years in the 1940's and 1950's were spent in the rural community of Peabody, Kansas that I still call home today (HWK). This is tall grass prairie country located in central Kansas on the edge of the Flint Hills. I enjoyed hunting, fishing, and outdoor activities with my friends and family but had no interest in biology or becoming a scientist. Our family had business interests that included clothing stores, farming, and ranching. I spent most of my time working in our clothing store (men's and boy's wear) selling brand name merchandise and making extra money with my shoeshine stand. I spent summers doing farm work using tractors to plow, disc, duck-foot and chisel and drove a self-propelled combine for custom cutting wheat and milo. I was also a "grease monkey" because these were still the days of grease zerts for moving parts of equipment, especially combines, which had to be properly greased every morning before the start of operation. Our trucking operations involved hauling cattle and swine with stock racks on the truck bed. Wheat and milo were hauled by truck to the nearest elevator or dumped on the ground using a hydraulic lift or corn silage taken to a conveyor and blown into a silo. My parents taught me hard work, honesty, and doing my chores were life lessons that would make a positive difference and a better person.

The Lolo National Forest in Idaho: becoming a botanist and mycologist

I (HWK) planned to major in Business Administration at Kansas Wesleyan University (KWU) but my father died during my freshman year at the age of 44 of a brain tumor. My life floundered without any sense of direction or self confidence. I applied for a job with the United States Forest Service and was hired to work as part of a trail crew in the Lolo National Forest (this area is now part of the Clearwater National Forest) assigned to the Powell Ranger District (Headquarters at Powell Ranger Station). This location is also noteworthy because it is near Powell Ranger Station along the Lochsa River where members

of the Lewis and Clark Expedition suffered from winter hardships and lack of game at their campsite on September 14, 1805 and today a sign records their presence with these words “compelled to kill a Colt…for the want of meat…” (Moore 1996).

The first summer during June 1958 I was part of a trail crew clearing trails along the road to Elk Summit Guard Station. Snow was still on the road and trees blocked the roadway and off-road trails. We used a crosscut and chain saw, a double bitted axe, and a Pulaski to clear mostly tree debris from the roads and trails. We had to wear silver hard hats for safety reasons and I wrote KANSAS in big black letters on the front. Nobody wanted to climb trees to re-splice and re-hang the telephone line so Lennie Smith our foreman asked me to do it. I did not tell him that I was afraid of heights and sometimes got vertigo but I said, “okay, I’ll give it a try.”

The telephone line often was broken and had to be re-pliced and re-hung 6.1 to 12.2 m off the ground. Our climbing gear consisted of a belt that was worn buckled around the waist and climbing spurs/gaffs strapped and buckled to the inside of the legs and around the instep of heavy boots. *Pinus contorta* Douglas ex Louden (lodge pole pine) was the tree usually used for hanging the telephone line. It had a straight trunk with few branches until about 6.1 m and relatively thin bark that spurs can easily penetrate. This type of climbing gear is often used by professional arborists, telephone pole climbers, and loggers. The puncture wounds from climbing spurs often resulted in sticky, resin droplets that exuded from the thin bark and covered the climber’s shirt and pants. This climbing method is not allowed in our national parks today.

The first summer I trained at Nine Mile Ranger Station north of Missoula, Montana where I learned to run a compass course to locate smoke and use an azimuth to record lightning strikes and fires. Most of the snow had melted at higher elevations by the first or second week of July and lookout observers were assigned to different locations. Bear Mountain lookout tower was elevated approximately 15.2 m above ground level anchored by poles and guy wires. It commanded a panoramic view of the area. I was assigned to Diablo Mountain Fire Lookout that was a cabin on the ground at the edge of a precipice but commanded a view to the east to Missoula, Montana, to the south through Blodgett Pass, and to the west a panoramic view of the Selway-Bitterroot Primitive (Wilderness) area. Norman Maclean’s book, *A River Runs Through It*, made into a popular motion picture, mentions this area and indeed he spent time at Elk Summit Guard Station and in the Bitterroot Mountains. This wilderness area has no passable roads and effectively has blocked any North–South highway through the central part of Idaho (Moore 1996). Diablo Mountain Lookout was at an elevation of approximately 2,274 m. The steep trail to Diablo Mountain had many switchbacks the last few miles hence the name Diablo in Spanish means devil. I stayed at Diablo Lookout for about two months spotting fires and recording lightning strikes. The second summer I was part of a trail crew building the Skyline Trail using dynamite to clear the area for a foot trail. We also were a smoke-chasing team that was on-call to fight fire in the area. Our home base was Elk Summit Guard Station. Hoo Doo Lake was a short 0.4 k hike and it was easy to catch native cut-throat trout, rainbow trout, and eastern brook trout fly fishing or with daredevil spoons. We could catch breakfast or supper in a short period of time.

These two summers of 1958 and 1959 spent in the Bitterroot Mountains and working for the forest service in the Lolo National Forest kindled my interest in forestry. About this same time I met Professor Albert Robinson, Jr. at KWU and took botany and mycology courses from him. My grades improved and my interest in plants was sparked by his encouragement that I go to graduate school at the University of Kansas and work with Professor Ronald L. McGregor, head of the Department of Botany. He wrote a letter of recommendation that resulted in my being selected as a graduate student teaching assistant in the General Botany laboratory classes. His confidence and support over the years made the difference in my success that led to a career path in science.

My research mentor and doctoral dissertation advisor at The University of Iowa (1967–1971) was Professor George W. Martin who instilled in me the passion to learn more about the taxonomy of the myxomycetes through his scholarly example. His journal papers and books published on the Myxomycetes (true or plasmodial slime molds) and Tremellales (the jelly fungi) set the highest standards of scholarship. His last book, a world monograph entitled *The Myxomycetes*, published in 1969 with C. J. Alexopoulos, is still considered the most authoritative work on the subject. This book and his lifelong work on the Myxomycetes were cited as the basis for the Henry Allan Gleason Award given in 1970 to Professor Martin by the New York Botanical Garden for an outstanding recent publication in the fields of plant taxonomy, plant ecology, or plant geography. Professor Martin was referred to as “The Boss” by many of his earlier students, but I just could not bring myself to do that since I considered him my second father. He presented me an autographed copy of his world monograph *The Myxomycetes* with this notation: “To Harold Keller, my last student, with sincere regard, and the hope that he will do much to improve this treatment.” This began my career as a myxomycologist.

Corticolous Myxomycetes, Cemeteries, and Red Cedars

Corticolous myxomycetes represent a group of species that are adapted to complete their life cycle only on the bark surface of living trees and vines. Most species that form fruiting bodies on living trees are tiny, less than 1mm, and difficult to see. The fruiting bodies form microscopic spores that survive in a dormant stage for many years. The spores germinate into microscopic motile stages such as myxamoebae that require moist conditions and/or flagellated, swimming swarm cells that require a thin film of water. These haploid motile stages encyst into a microscopic dormant stage called a microcyst. The diploid plasmodial stage may be microscopic or several centimeters or sometimes several meters large, brightly colored, and may form a dormant, usually visible stage called a sclerotium that survives unfavorable environmental conditions (Keller and Braun 1999, Everhart and Keller 2008). Other myxomycete species complete their life cycle on ground sites such as decaying logs and leaf litter in temperate and tropical forested areas, high mountainous regions under snow-banks, grasslands, semi-arid desert areas on decaying xerophytic plants, and usually associated specifically with these habitats and substrata when adequate moisture is present. These myxomycete species usually form larger fruiting bodies in greater numbers covering a wider area and are more conspicuous to the unaided eye (Keller and Braun 1999, Everhart and Keller 2008).

Credit must go to Dr. Travis E. Brooks who acquired in the 1960’s special field knowledge such as the time of year, rainfall, temperatures, and species of

tree to locate and collect the corticolous myxomycetes (Keller 1979). He shared his collecting field experiences and observations with me suggesting that the living Eastern Red Cedar tree, *J. virginiana*, was one of the most productive trees in species richness of coricolous myxomycetes (Keller 1996, Keller and Braun 1999).

Cemeteries located throughout the central and southeastern United States of America often had *J. virginiana* trees, sometimes to the exclusion of other tree species. Apparently *J. virginiana* was planted near gravesites because it was readily available as a native tree species, it grew well in open field areas in full sun, and symbolically was considered the "death tree" (Keller and Braun 1999). Field forays for corticolous myxomycetes in the states of Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Ohio, Oklahoma, Tennessee, and Texas were often at cemetery sites with *J. virginiana* trees. Much more time was spent collecting in Ohio, Florida, Arkansas, Kansas, and Kentucky in approximately that order. How did we locate cemeteries with red cedars? Smaller communities and towns usually had some older person on the main street, who when asked the question about the location of old cemeteries, would give directions to one or two cemeteries within the city limits and also near churches in the surrounding rural areas. Cities with larger populations and more urban sprawl required using the yellow pages of the telephone directory and looking under cemeteries to get the exact street address.

Ideal conditions of a soaking rain, cloudy weather that keeps the bark of living red cedars moist for several days, and warm summer temperatures generally from mid-June to the end of September, resulted in myxomycete fruiting bodies that were collected at 1.5 to 1.8 m on the bark surface of trunks. My first experience collecting from *J. virginiana* trees was while I was in residence (1971-72) at the University of Florida, Gainesville on a Postdoctoral Fellowship awarded by the Graduate School based on a nationwide competition. My project proposal was to study Myxomycetes throughout the state of Florida and to do transmission and scanning electron microscopic studies with Professor Henry C. Aldrich in the Department of Botany and correlate ultrastructure with myxomycete taxonomic problems. Evergreen Cemetery, located within the city limits of Gainesville, was at 401 SE 21st Avenue, a short drive from the University. The first myxomycete collections were made July 28, 1971 and the last collections November 21, 1971 and the next year the earliest collections were made June 24, 1972. Species of myxomycetes developed fruiting bodies much later in the year than farther north (HWK per. obs.) apparently due to the warmer temperatures in this region of north central Florida. This part of Florida is north of the frost free zone evidenced by several hard freezes of approximately 6.7°C. *Juniperus virginiana* trees were numbered (more than 200) to pinpoint the location of myxomycete species that occurred on each tree. The majority of the trees were *J. virginiana* with only a few pines that lined the roadway from the main entrance. The first paper published in a series of related papers on corticolous myxomycetes (Keller and Brooks 1973) cites collections of *Didymium orthonemata* H.W. Keller & T. E. Brooks on 20 *J. virginiana* trees (sometimes referred to as *J. silicicola* (Small) Bailey supposedly a southern species).

The history of the Evergreen Cemetery in part accounts for the great number of *J. virginiana* trees on the grounds. The first grave plot recorded in February of 1856 was near a young *J. virginiana* tree (Dr. Thomas H. Fay pers. comm.). Many red cedar trees were planted since then but wind storms and prolonged

drought conditions have taken their toll on the number of these majestic old trees (Fig. 1). Two myxomycete species developed extensive and conspicuous fruiting bodies visible to the unaided eye, for example, the sessile, white, mostly plasmodiocarpous habit of *Didymium orthonemata* and the brownish, stalked, clustered sporangia of *Stemonitis flavogenita* E. Jahn on 19 *J. virginiana* trees. These two species could be seen from ground level fruiting at higher points in the tree canopy. Several *J. virginiana* trees were climbed free-handed using the lower horizontal branches to follow myxomycete species that had fruited along the trunk axis vertically higher in the treetop. Other tiny species of the genus *Licea* were abundant in the tree canopy, for example. *Licea denudescens* H.W. Keller & T.E. Brooks was a species described new to science with the holotype locality in Evergreen Cemetery (Keller and Brooks 1977).

FIG 1. Photograph of *Juniperus virginiana* at Evergreen Cemetery, Gainesville, Florida, October, 2008.

Field observations of *Arcyria cinerea* occurred on many of the *J. virginiana* trees in Evergreen Cemetery but only a few collections were made because of the widely scattered and solitary habit. Sporangia on living trees were the typical single, stalked sporangium in contrast to the more gregarious habit and digitate form that develops extensive fruiting bodies found on decaying wood or logs on ground sites (pers. obs. HWK).

A list was compiled from mostly field-collected myxomycete species on the bark surface of living *J. virginiana* trees and also harvested from moist chamber bark cultures. This species list (Table 1) was made over a period of at least 35 years and multiple trees (approximately 250 trees). There was at least one species (a spiny reticulate-spored *Stemonitis*) found on many trees in Florida's Evergreen Cemetery that is still unidentified. Bark from most of these trees was collected at a height of 1.5 to 2 m. One species, *Diachea arboricola*, was collected only from the tree canopy above 2 m. Taxa are arranged alphabetically

by order and genus and nomenclature generally follows Martin and Alexopoulos (1969).

Table 1

Myxomycete species from multiple trees of living *Juniperus virginiana* (Eastern Red Cedar) organized by taxonomic orders

Order Echinosteliales

Echinostelium arboreum H.W. Keller & T.E. Brooks +, *E. coelocephalum* T.E. Brooks & H.W. Keller +, *E. elachiston* Alexop.+, *E. fragile* Nann.-Bremek.+, *E. minutum* de Bary+, *Clastoderma debaryanum* A. Blytt var. *emperatorium* Emoto+, *C. microcarpum* (Meyl.) Kowalski+, *C. pachypus* Nann.-Bremek.+ (8 taxa).

Order Liceales

Licea biforis Morgan*, *L. denudescens* H.W. Keller & T.E. Brooks**, L. inconspicua* T.E. Brooks & H.W. Keller*, *L. kleistobolus* G.W. Martin*, *L. nannengae* Pando & Lado**, L. operculata* (Wingate) G.W. Martin*, *L. parasitica* (Zukal) G.W. Martin*, *L. pedicellata* (H.C. Gilbert) H.C. Gilbert* , *L. perexigua* T.E. Brooks & H.W. Keller*, *L. pseudoconica* T.E. Brooks & H.W. Keller*, *L. scyphoides* T.E. Brooks & H.W. Keller*, *Licea* sp.* (unidentified and unnamed); *Cribraria minutissima* Schwein.*, *C. violacea* Rex*, *Dictydiaethalium plumbeum* (Schumach.) Rostaf.*, (15 taxa).

Order Physarales

Badhamia affinis Rostf.*, *Badhamia rugulosa* T.E. Brooks & H.W. Keller*, *Badhamiopsis ainoae* (Yamash.) T.E. Brooks and H.W. Keller*, *Diachea arboricola* H.W. Keller & M. Skrabal*, *Diderma corrugatum* T.E. Brooks & H.W. Keller**, D. chondrioderma** (de Bary & Rostaf.) G. Lister**, Didymium clavus* (Alb. & Schwein) Rabenh.**, D. orthonemata, D. synsporon* T.E. Brooks & H.W. Keller**, Physarum aeneum* (Lister) R.E. Fr.+, *P. auriscalpium* Cooke*, *P. crateriforme* Petch**, P. nutans* Pers.*, *P. synsporum* S. L. Stephenson & Nann.-Bremek.* (should be reassigned to the genus *Badhamia*), *Trabrooksia applanata* H.W. Keller*(15 taxa).

Order Stemonitales

Comatricha cf. *laxa* Rostaf.*, *Macbrideola cornea* (G. Lister & Cran) Alexop.*, *M. declinata* T.E. Brooks & H.W. Keller*, *M. decapillata* H.C. Gilbert*, *M. scintillans* H.C. Gilbert+, *Stemonitis flavogenita* E. Jahn*, *Stemonitis* sp.* (unidentified and unnamed with spiny-reticulate spores) (7 taxa).

Order Trichiales

Arcyria cinerea (Bull.) Pers.**, Dianema sp.* (unidentified and unnamed)*, *Calomyxa metallica* (Berk.) Nieuwl.**, Minakatella longifila* G. Lister**, Perichaena chrysosperma* (Curr.) Lister**, P. depressa* Lib.*, *P. minor* (G. Lister) Hagelst. var. minor*, *P. minor* var. *pardina* (Minakata) Hagelst.**, Trichia* cf *contorta* (Ditmar) Rostaf.* (9 taxa).

Total number of different myxomycete taxa from bark of living *J. virginiana* trees=54

*field collections from living trees and + specimens harvested from moist chamber bark cultures

The data in Table 1 suggests that *J. virginiana* represents a tree species with unusually high species diversity of myxomycetes. There are several reasons that this may be a subject to question. For example, comparing the data in Table 1

with data from Everhart et al. (2008), who sampled primarily from GSMNP and the Daniel Boone National Forest (DBNF) in Kentucky, show that collections from *J. virginiana* were from a larger geographic region. Second, the collections from *J. virginiana* were from approximately 250 individual trees, whereas the species-area curve represents 60 individual trees sampled (Figure 3). Third, samples from *J. virginiana* were taken from multiple localities that were not in the same habitat type, including parks and urban areas in different states.

There are several possibilities why *J. virginiana* has such high myxomycete species diversity. Records from *J. virginiana* are largely from field collections which are an underestimation of the total number of species, since many species remain dormant, developing fruiting bodies only under optimal conditions (such as in moist chamber cultures) for a very brief period of time and are fragile and easily destroyed. In addition, many species are rarely observed as field collections because their fruiting bodies are extremely tiny, less than 1 mm, and only visible with directed light from a dissecting microscope (Keller and Braun 1999, Everhart et al. 2008). Therefore, the total number of species based largely on field observations may be an underestimation, suggesting *J. virginiana* has even more species than those listed here. *Juniperus virginiana* has a bark pH that is nearly neutral (Table 2) and therefore is able to host myxomycete species that are pH generalists and marginally support both acidophilic and basophilic species. This near neutral pH would therefore optimize myxomycete species diversity.

Macroscopic characteristics of the bark, tree architecture, and environmental conditions would also favor high myxomycete species diversity. The bark is spongy and highly water absorbent and the lower trunk of the tree is usually exposed with the branching beginning at approximately 1/3 of the total height of the tree. With the lower portion of the tree trunk exposed, rain would readily soak the highly water-absorbent bark of the tree. *Juniperus virginiana* is also commonly planted in cemeteries and invades open field habitats (it is not shade tolerant and only occurs at the margins of forested areas), therefore windblown spores of myxomycetes would more readily adhere to the bark surface and complete the life cycle. Thus, after a rain, high air temperatures and high moisture content would favor myxomycete growth on the exposed trunks of *J. virginana.* Fewer myxomycetes would develop under a dense forest canopy which would not warm as quickly and retain humidity on the tree trunks – two factors which decrease myxomycete development and maturation. These environmental factors associated with *J. virginiana* trees would appear to select and favor the rapid development of mature myxomycete fruiting bodies under natural conditions. This rapid cycle of myxomycete development and sporulation, often between 24 to 72 hours, would reduce competition in the plasmodial phase thus providing more niche space for many more corticolous species (Everhart and Keller 2008).

Tree Canopy Biodiversity in the Great Smoky Mountains National Park

A grant proposal to the National Science Foundation Small Grant for Exploratory Research through the Biodiversity Surveys and Inventories Program was based on the application of a new approach to explore the tree canopy for cryptogams (myxomycetes, macrofungi, mosses, liverworts, lichens, and ferns) using rope climbing methods. The seminal idea was based on previous experiences using free hand climbing methods exploring and collecting

myxomycetes vertically along the trunks of living *J. virginiana* trees. The cryptogams were in the tree canopy so we utilized the best and safest way to climb the tree to gather samples along vertical transects.

The Doubled Rope Climbing Method (DRCM) was used in this project because it was more of a noninvasive method and would minimize any injury to trees compared to the pole climbing spurs that were used in the past (Kilgore et al. 2008). Scientific Research and Collecting Permits issued by the United States Department of Interior National Park Service for the GSMNP required details about the climbing protocol that included collecting methods, safety precautions that ensured students were qualified tree climbers, and adequate preparation for any emergency situation. Safety precautions were always emphasized as a priority during the course of this tree canopy project. Fortunately, Charly Pottorff, a professional arborist from Manhattan, Kansas, had a special interest in this project from the beginning and served as the instructor for our tree climbing schools held at Pertle Springs, Warrensburg, Missouri. His professional experience climbing champion-sized trees and certification of mastering the DRCM was invaluable when teaching the use of the Big Shot (an over-sized sling shot to get a throw line in the tree canopy), the basic knots used for the DRCM, the proper use of climbing saddles and safety lanyards, and the strength, agility, and advancing methods to reach 30 m or to the treetop. Details about the climbing school, the DRCM, and student research experiences (the Adventure Phase, Laboratory Phase, and Publications Phase), were described by Counts et al. (2000), Keller (2004) and Kilgore et al. 2008. Additional papers that included how the sampling protocols and data were gathered for different tree species were discussed in Snell and Keller (2003), Snell et al. (2003), Keller (2004), Keller (2005), Everhart et al. (2008), and Kilgore (2008).

Influence of Bark pH on the Occurrence and Distribution of Tree Canopy Corticolous Myxomycetes

The first tree canopy study that used the DRCM examined the vertical distribution of corticolous myxomycetes that grow and fruit on the bark surface of living trees, including any possible new species and new records in the GSMNP (Snell and Keller 2003). Bark samples were collected at 3.3 m increments, transported to the laboratory, and prepared in moist chamber cultures consisting of sterile, polystyrene, round, Petri dishes (150 X 25 mm). Each Petri dish was fitted with filter paper and the bark placed face up covering the bottom of the Petri dish. The bark was wetted with 35 mL of sterile, de-ionized water adjusted to a pH of 7. After 24 h excess water was decanted and pH was measured in three random places on the filter paper near or under bark pieces with an Orion model 610 flat probe pH meter. Moist chambers were cultured in ambient light and room temperature (23.-25 C.) These moist chamber cultures were scanned for myxomycetes on day 4, 8, 16, and 32. The scanning of bark cultures, recording species present, preparing voucher specimens, and identification was a time consuming process estimated to take 15 to 30 min per plate. This limited the number of moist chamber cultures that could be examined during a two years Master's Degree Program (420 cultures in Snell (2002), Snell and Keller (2003), 580 cultures in Everhart (2007), Everhart et al. (2008). Tree canopy myxomycetes were represented by 95 different species, including 52 new records for the Park based on 209 bark samples from 25 trees of 5 different tree species (Snell and Keller 2003, Snell et al. 2003). Since then 10 new

myxomycete records from ground sites and two new records from the tree canopy have been found with a total number of species for the Park approximately 220 (Keller 2004, Everhart et al. 2008). Although there was no relationship between species assemblages and height in the tree canopy, 30 myxomycete species or 32%, were known only from the tree canopy and not found on ground sites (Keller 2004).

The use of Sørenson's coefficient of community index (CC) showed that the CC of *Pinus strobus* L. and *Fraxinus americana* L. were significantly different (Snell and Keller 2003) and these two tree species were at opposite ends of the pH scale (Table 2). Additional data showed that the community of myxomycetes was consistent among trees of the same species and was associated with bark pH. Five tree species were measured at diameter at breast height (d.b.h.-1.3 meters) and also for total height with a measuring tape carried by climbers. These five tree species were all a minimum of 27 m in total height and the range in d.b.h. was from 40 to 180 cm. The range in d.b.h. is given in cm with (parentheses) for each tree species *Acer rubrum* L. (40- 83), *Fraxinus americana* L. (52-104), *Liriodendron tulipifera* L. (80-180), *Pinus strobus* L. (69-91), and *Quercus alba* L. (51-90) in Snell and Keller (2003) are shown in Table 2. *Acer rubrum* had the highest species richness (Table 2) and mature plasmodiocarpous fruiting bodies of the myxomycete *Hemitrichia serpula* (Scop.) Rostaf.were obtained in the field on more flaky bark 20 m above ground level on the inner bark surface. This myxomycete typically is found on mixed leaf, twigs, small decaying limbs, and the underside of well decayed logs on ground sites. The canopy trees were preselected for their larger size and apparent older age as evidenced by bark characteristics, d.b.h., and total height, and also when combined with extremely wet conditions, that had resulted in typical ground site myxomycete species in the upper tree canopy (Snell and Keller 2003).

These results were substantiated with a follow-up study that examined 290 bark samples from 30 trees of 6 species and 30 grapevines of two species that neighbored these trees (Everhart et al. 2008). Thus, it was important to conduct large scale studies on the corticolous myxomycetes with many bark samples because the distribution of myxomycetes was not even and often patchy over bark surfaces of living trees. In addition, Everhart et al. (2008) showed that environmental factors changed bark pH and subsequently yielded a species assemblage of corticolous myxomycetes that was characteristic of different tree species that had similar pH. Further studies showed that different plant parts on the same gymnosperm tree (bark and cones) also yielded different pH values and different species assemblages (Kilgore 2008).

Tree Species pH with Associated Myxomycete Species Assemblages

More than 500 trees have been climbed and sampled during the course of this project but not all were part of the moist chamber cultures and the pH measurements reported here. The mean and standard error were found by converting pH values on the logarithmic scale to linear scale hydrogen ion concentration. Once the mean hydrogen ion concentration was found, the value was converted back to the logarithmic pH value. In order to calculate standard error, the mean pH value had to be subtracted from the –log [SE H+]. This ensured that no rules were violated on calculating mean values from a logarithmic scale (Table 3).

Table 2

Summary of tree species for pH, moist chamber cultures, and number of myxomycete species.

Tree Species	No. of trees	pH ± SE	No. of cultures	No. of pH readings	Total myxo. sp.
Picea rubens	4	3.7 ± 0.05	32	69	10
Pinus strobus	5	3.8 ± 0.16	86	258	24
Pinus echinata	8	3.9 ± 0.88	170	680	14
Abies fraseri	4	4.1 ± 0.06	32	51	0
Tsuga canadensis	5	4.1 ± 0.08	50	200	17
Acer rubrum	5	4.7 ± 0.14	72	216	49
Liriodendron tulipifera	5	4.9 ± 0.22	90	270	39
Platanus occidentalis	5	5.1 ± 0.04	48	192	10
Acer saccharum	5	5.5 ± 0.05	46	138	17
Quercus alba	5	5.7 ± 0.46	82	246	41
Liquidambar styraciflua	5	5.8 ± 0.04	90	360	10
Cercis canadensis	4	6.3 ± 0.51	64	256	11
Juniperus virginiana	6	6.7 ± 0.09	50	177	50
Fraxinus americana	5	6.7 ± 0.16	86	258	31
Total 14	71	n/a	998	3371	n/a

Table 3

Summary of mean pH ranges and associated myxomycete species assemblages

Mean pH 3.7 – 4.1

Cribraria confusa, C. rufa (Roth) Rostaf.*, C. ellae* Härk*, C. nigra* (Pers. ex J.F. Gmel.) J. Schröt.*, Enerthenema papillatum* (Pers) Rostaf.*, Physarum nutans*

Mean pH 4.2 – 5.8

Badhamia rugulosa, *Clastoderma debaryanum* A. Blytt, *Clastoderma pachypus, Comatricha acanthodes* Alexop., *Echinostelium coelocephalum, E. apitectum* K.D. Whitney, *Lamproderma biasperosporum* Kowalski*, Physarum crateriforme,*

Mean pH 5.9 – 7.0

Badhamia affinis, Cribraria violacea, Diachea arboricola, Didymium clavus, Licea biforis, L. parasitica, Macbrideola cornea, M. decapillata, M. declinata, M. scintillans, Trabrooksia applanata,

Mean pH 3.7 – 7.0

Arcyria cinerea, Calomyxa metallica, Diderma chondrioderma, Echinostelium minutum, Licea kleistobolus, Perichanea chrysosperma

Myxomycete species were placed with their respective tree hosts and were then grouped according to taxonomic order. The mean pH value for each group of host trees was calculated and these included the number of individual trees in (parentheses): *Abies fraseri* (4), *Cercis canadensis* (4), *Fraxinus americana* (10), *Juniperus virginiana* (6), *Liriodendron tulipifera* (10), *Pinus echinata* (8), *Picea rubens* (4), *Pinus strobus* (5), *Quercus alba* (5), and *Tsuga canadensis* (5) (Table 2 and Figure 2). The mean pH and standard error for the host trees of each species of myxomycetes was calculated and this was considered the mean pH range for that particular species of myxomycete. The myxomycetes were then grouped according to order with the number of different myxomycete taxa for each order: Liceales (21), Stemonitales (22), Physarales (32), Echinosteliales (9) - and the mean pH and standard error values were taken for a particular order.

Myxomycetes occur throughout a wide range of the pH scale (3.7 to 6.7) shown here for the tree canopy trees (Table 2) and even higher pH values for plants that occur in desert areas (Schnittler 2001) with a pH range of 7.3 to 8.7, mostly basophilic. Futhermore, plant substrata from arid areas in general ranges from a pH of 6.0 to 10.4. and pH of decomposing cactus debris had a high pH between 7.0 to 10.0 (Novozhilov et al 2006). Members of the myxomycete Order Stemonitales typically do not occur at these higher pH values and some species were restricted only to the lower pH values 3.7 to 4.1, and are therefore considered acidophilic (Table 2, 3). Examples of acidophilic myxomycete species in the Stemonitales are listed in Table 3.

The genus *Macbrideola* has been classified in the Order Stemonitales (Martin and Alexopoulos 1969, Keller and Braun 1999) without taxonomic controversy. In contrast, four species included in the genus *Macbrideola, M. cornea*, *M. decapillata*, *M. declinata*, and *M. scintillans* occur with great frequency on the bark of living canopy trees, *Fraxinus americana* and *Juniperus virginiana* (Snell and Keller 2003, Keller 2004, Everhart et al. 2008) and on shrubs in semiarid areas of New Mexico (Keller pers. obs.). This group of species occurs at the highest end of the pH scale (6.7) and obviously raised the pH values for the Order Stemonitales. Members of the genus *Macbrideola* have not been grown from spore to spore on agar culture nor has the plasmodium ever been observed as either a protoplasmodium or an aphanoplasmodium. The morphological characteristics exemplified by the translucent, hollow stalk without any enclosed debris seems out of place when compared to other taxa in the Order Stemonitales. Species of *Macbrideola* should be targeted for DNA analysis to determine their phylogenetic relationship to other genera such as *Stemonitis* and *Comatricha*.

Certain species of *Licea* and *Cribraria, C. confusa,* also show a trend toward a lower pH of 4.6 as does the Order Liceales (Fig. 2) but *Cribraria violacea* was found more frequently on the highest pH 6.7 trees (Table 1,2,3). Members of the Order Physarales exhibited a mean pH of 4.7, the Trichiales had a mean pH of 4.8, and the Echinosteliales had a mean pH of 4.9 (Fig. 3) which fall in the middle of the pH range. Most corticolous myxomycetes on the bark of living trees develop and form fruiting bodies mostly in the middle pH range of 4.2 to 5.8. Bark of all of these tree species was acidic with a pH of 3.7 to 6.7 (Table 2).

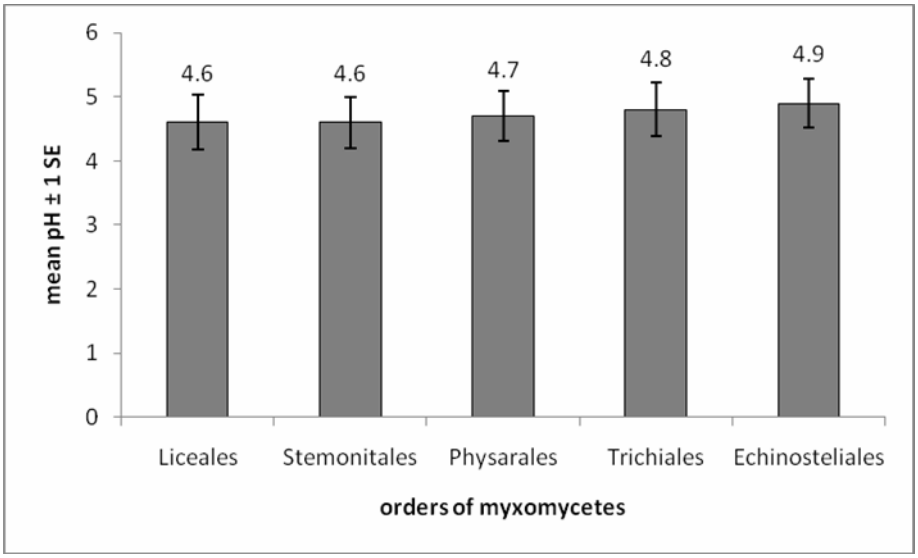

FIG. 2. Mean ± standard error pH for Myxomycete species grouped by taxonomic order.

Species-area curves (also called species-accumulation curves) are used to evaluate the adequacy of sample size in a community data set. The upper curve represents the average number of species accumulated by the number of sample units surveyed, which is calculated by taking a random sub-sample of the dataset for any given number of sample units and repeated 500 times (Figure 3). The lower curve utilizes Sørenson's distance-based algorithm that calculates similarity between these sample units in a similar fashion as the upper curve.

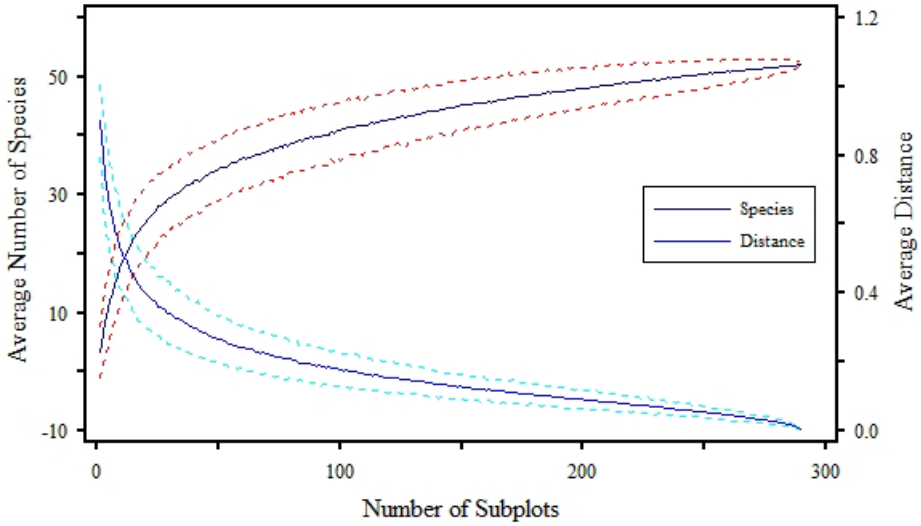

FIG. 3. Species-area curve showing accumulation of corticolous myxomycete species richness based on the number of subplots used, where 45 species are obtained at 50% sampling effort and 52 species accumulate at 100% sampling effort (290 bark samples, Everhart et al. 2008).

A species-area curve of 290 sample sites, constituting 6 tree species and 2 grapevine species, and 52 myxomycete species is shown in Figure 3 based on Everhart et al. (2008). Host species included five individuals of six tree species, *Acer saccharum*, *Fraxinus americana*, *Liquidambar styraciflua*, *Liriodendron tulipifera*, *Platanus occidentalis* and *Tsuga canadensis*, and neighboring grapevines, *Vitis aestivalis* and *V. vulpina*. The list of myxomycete species can be found in Everhart et al. (2008). Each sample site represents the presence of a myxomycete species from two over-sized moist chamber cultures of bark sampled from a single height of 3, 6, 9, 12, or 15 m from all sides of the trunk or grapevine.

This species-area curve begins to plateau after 50 samples with 34 species found and was consistent with species-area curves generated for myxomycete species in temperate forests (Unterseher et al. 2008). Beyond 50 samples, 18 more species are found only after increasing the sample size by approximately six-fold. Therefore, sampling beyond 50 samples increases the total species found by 35%, sampling beyond 100 samples increases the species list by 21% (11 species), sampling beyond 150 increases the species list by 13% (7 species), and sampling beyond 200 species increased the species list by 8% (4 species). Total species richness for this dataset was estimated to be 63 species with a first-order jackknife and 69 species with a second-order jackknife procedure.

Large-scale studies of myxomycetes also aid in evaluating the role that species-specific and intra-specific relationships play in community composition. For example, previous studies have shown that there are species-specific associations between certain myxomycete species and perennial epiphytes, such as lichens, and bryophytes (Gray and Alexopoulos 1968, Ing 1994, Keller and Braun 1999, Novozhilov et al. 2000, Schnittler et al. 2000, Smith and Stephenson 2007). Nevertheless, examining community associations among all corticolous species and the presence of other epiphytes has yielded weak associations (Everhart 2007). These results demonstrate that the occurrences of myxomycetes are largely independent of other epiphytes. The majority of these large-scale projects have indicated that community partitioning of myxomycetes does not go far beyond associations with bark pH/tree species.

A New Tree Canopy Myxomycete Species: An Undergraduate Student's Perspective

The personal narrative found in this topical section is by Melissa Skrabal entitled "My Eyes Opened and There It Was!" that described her field and laboratory discovery of a new myxomycete tree canopy species *Diachea arboricola* H.W. Keller & M. Skrabal (Keller 2004; Keller and Skrabal 2002; Keller et al. 2004). This new species apparently represents the first arboreal (upper canopy) myxomycete recorded to date and is only found in the Great Smoky Mountains National Park. This was part of an undergraduate student research project at UCM (Keller 2005). She graduated August, 2001 from UCM with *Magna Cum Laude* honors.

Melissa begins: I awoke to a morning dawn that could not have been more beautifully intense. Remembering that today was our first day to test the Big Shot my mood began to change for the best as I hustled around in my best attempt to prepare for the upcoming day. This Schwarznegger-sized tall slingshot (2.4 m) was supposed to help save time and effort for hoisting our throw line over the first crotch of a tree. Why throw ropes up into the trees you may be asking? This tree

canopy biodiversity research project was part of the All Taxa Biodiversity Inventory (ATBI) research project that took place in the Great Smoky Mountains National Park. This project was designed to record all of the living organisms in the Park. Our part of the ATBI project took place in the treetops which had never been explored before.

Each crew member greeted me as I elbowed my way through the crowded kitchen in order to get a measly bowl of cereal. Some of my fellow climbers "good mornings" were of high-pitched cheer while others were less enthusiastic. After ten days of this routine we had learned how to cut our preparation time down to a bare minimum. Our climbing gear remained packed in the vehicle from the last day's excursion so all the time left was to prepare our lunches. More comfortably packed than sardines, we crammed into the van and drove to our destination. Luckily the drive to Turkey Pen Ridge Trailhead was only about ten minutes from our Cades Cove house. Slowly getting out of the van the student climbers gave one last stretching effort to wake up and then unpacked our gear. We slung our seventy-pound gear bags onto our backs (each comprised of a harness, 36.6 m climbing rope, altitude line, gloves, and more) and began to hightail it up the trail. Buck Counts had the "Big Shot," tied onto his backpack in such a fashion that required a *WIDE LOAD* sign to be glued to his backside. Our ground crew loaded up their arms with logbooks, safety hats, and bug spray. Even though the trail was a fairly easy incline covered with small rocks and horse dung, the weight of our gear bags made the hike a more challenging jaunt.

Now fully awake, marginally from the shock of the crisp morning air, and mainly due to the sudden caffeine jolt thanks to the morning's coffee binge, the climbers happily shared gossip and jokes as if we were back in junior high. After hiking slightly more than a mile we came to a fork in the trail. We could not decide whether to stop and climb or continue on but most of us were impatiently itching to climb and test the "Big Shot". We stopped and scouted out the surrounding territory. Very unimpressed with the size of the trees, I was convinced that we would not have a very productive climb. I figured we would be up and down the trees in no time and with little accomplished due to their tiny sizes of approximately 30 m tall.

Breaking out the bright yellow Big Shot we gave it a test run on a white oak (*Quercus alba*). It worked marvelously. The job of precisely lassoing the first high branch of a tree (usually about 18 to 21 meters up) used to take 30 frustrating minutes using a hand throwing technique, but now it took only about five with the Big Shot. The throw line had never been hurled so accurately and gracefully in such record-breaking time. Selfishly hoping for a more robust tree, I harnessed up and secured the climbing line and grudgingly began the ritualistic climbing procedures. Coordinating all my body parts in a rhythmic upward motion and incorporating intense effort from my back and upper body muscles, my ascension began. Synchronized pull-ups and pelvic thrusts enabled me to reach a height of 6 m where I momentarily paused to nail an identification number to the tree (#88). After securing the laminated number with a nail, I carefully removed some of the tree's outer bark layers using a large-bladed knife. After filling a small white paper bag half full with bark, I folded the top edge over and let it cruise to the ground for Ted Stampfer to label with the appropriate data (tree number, height of collection, and climbing site). Collection height was measured using an altitude line that was attached to the harness and hung down to the ground. These handy

lines were handmade by our ground crew by attaching labeled paper tags to a lightweight line.

Climbing higher and higher to the rhythm of my own song, I paused once again at about 9.1 m to take another bark sample. During my usual scanning of the bark before removal I noticed an unusual snake-like pattern on the bark surface. Suddenly vivid flashbacks of Dr. Keller's slide show on the myxomycete life cycle appeared in my mind. The previous night Dr. Keller presented a lecture to all of the climbers and ground crew in order to orient our eyes for recognizing Myxomycetes. As an insecure undergraduate student and non-myxo expert, I thoroughly doubted what I was seeing (on the tree, not in my mind). The tracks on the bark looked identical to the remains of plasmodial tracks left by developing myxomycetes. In a bewildered state my voice cracked as I hollered down to Ted Stampfer about these weird configurations. Ted and I continued on, as Dr. Keller remained faithfully at his station helping another climber under a different tree up the trail. Meticulously, I scanned the circumference of the tree with my naked eye. I then found tiny myxo-like sporangia scattered among the side-winding plasmodial tracks. Taking another large sample and sending it down to Ted, I nonchalantly requested that Dr. Keller come have a look at my discoveries upon completing his task.

Inching my way higher up, I paused at 3.3 m increments to collect more bark samples. Oddly, the twisted pattern on the bark seemed to follow my ascent up to almost 26.4 m. Dr. Keller was finally freed of his duties and took the opportunity to glance at my samples using a hand lens. Swaying high above the muddy forest floor I heard a lot of hooting and hollering like it was a Fourth of July celebration and I saw Dr. Keller do the jitterbug dance (ironically, the day was the Fourth of July, 2000). The celebration below me confirmed the discovery of a very unusual myxomycete. I enjoyed the free roller coaster ride of swaying to and fro in the tree top in the peaceful wind and in the panoramic view of heaven. I was eager to come down because the harness began cramping my legs. The day's heat index continued to skyrocket as I hung in discomfort. Dr. Keller enthusiastically asked that I come down in smaller steps and sample more bark about every three meters.

Finally, after about two hours up in the treetop I reached solid ground. Releasing the tight grips of the harness and shaking out my numb legs, I took a deep breath and smiled at the crew helping me. The quickly passing morning gave way to hunger pains so knowing there was still a lot more climbing ahead that day, we all sat down to devour our lukewarm sack lunches as the new discovery hibernated in its collection bag. Glories die fast when you have a hungry stomach.

After lunch our crew continued the hike up Turkey Pen Ridge Trail, which was now at a more rigorous incline. Then, all of a sudden, we came to a steep drop-off that opened up to a large cave entrance with a magnificent waterfall cascading down into its depths. Sliding and slowly scooting we cautiously went down the ledge. The temperature fell about ten degrees and soothed our overheated pulses. Laura Henley and I climbed two nearby walnut trees while the others reaped the comfort of the surrounding elements. After the passing of another two hours the gang packed up and made a beeline back to the car.

We drove home and upon immediate arrival at our humble abode we threw some hardy supper on the grill. Anxiously, I dragged out the samples of *Q. alba* tree number 88 and began to separate out the day's unusual discovery.

Dr. Keller was extremely impressed as he scurried around boxing up some of the more impressive samples. Finally, the food was ready and we had to be pried away from the beautiful display of Myxomycetes. With bark flakes deeply engrained into our hard-working hands, we happily rejuvenated ourselves with grilled chicken, potatoes, and mixed vegetables. Without any real jaw-dropping firework display, we truly had ourselves a very explosive Fourth of July. The day's spectacular discoveries and accomplishments helped us sleep well and look forward to the final two days of our three-week trip.

By the end of our first three-week research session in the Great Smoky Mountains National Park, we had sampled from 80 to 90 trees of various kinds: white oak, ash, tulip poplar, American elm, eastern red cedar, sweet gum, white pine, walnut, and more. During our second, three-week excursion we sampled from approximately the same amount of trees. At the end of each climbing session, ample time was taken each evening to divide up each tree's sample bags into different categories of organisms. The categories included liverworts and mosses that were taken by Dr. Paul Davison, liverwort expert, and Dr. David Smith, moss expert; the lichen category which went to Dr. Alex Ciegler, lichenologist; and the moist chamber category which the students took back to UCM for our own research purposes. A portion of the white oak tree number 88 sample was given to Professor Uno Eliasson from Sweden and Dr. Ted Stampfer, moist chamber culture specialist, for further investigation of the new myxomycete discovery. Even after appropriate separation, the sample bags were so numerous that they barley squeezed into the back of a Toyota pickup truck. Completing our collection task for the summer of 2000, our climbing crew returned to Warrensburg, Missouri, to begin moist chamber cultures and simultaneously start the fall school session.

This was my first undergraduate field research experience. I did not exactly know where to start with the overwhelming number of bark specimens. I was especially intimidated by all of the new information of how to set up moist chamber cultures (consisting of a Petri dish, sterile filter paper, properly arranged bark pieces, and the necessary amount of autoclaved water), harvest the developed sporangia, label and categorize the species, take digital pictures of the myxomycete developmental stages, and make slides in order to take precise measurements and notes on the morphology of spores, capillitial threads, stalk, columella, and more. Because Dr. Keller had warned us from the very beginning that this second phase of our research project would not be as exciting as the climbing endeavor, I prepared myself for a monotonous boring semester of research.

I grew to enjoy the laboratory aspect of seeing the diverse beauty of life and harvesting the developmental stages of myxomycete sporangia. For several weeks the experts, Drs. Uno Eliasson and Ted Stampfer, had been trying to get the new myxomycete species of *Diachea* to grow in culture, but neither of them had been successful. Surprisingly one of my moist chambers containing bark from tree 88 developed the growth of a magnificent yellow plasmodium. My boredom changed to excitement when observing the development of myxomycete plasmodia and sporangia in moist chamber cultures each morning. The day was an ordinary school day starting at eight in the morning. I casually walked to our research laboratory while smiling at passing friends. I sat down for the millionth time to scan the moist chamber cultures with a dissecting microscope; when low and behold an iridescent gold peridium attached to a

reddish-orange stalk came into view. I did not debate very long whether or not to call Dr. Keller's private "emergency only" hotline. Unconvinced of my findings Dr. Keller continued on with his own daily professional work. My bubble of excitement burst so I dragged myself to my nine o'clock class. After class I retreated back to the research room to see if Dr. Keller had visited the premises. Upon my arrival I saw Dr. Keller doing the jitterbug once again and saying phrases like "Oh, my word! I didn't believe you. How did you know it was the new species?"

Our whole research project from the very beginning has been much like a fairy tale. Our undergraduate crew was interviewed by several newspapers and we have written numerous magazine articles. The publicity is nice but that is not what really matters. The priceless discovery of the deep beauty of nature is what really makes things count. I never knew such glory existed in microscopic proportions. It is truly a shame that so little of the world's population will ever see these splendid organisms. This project has taught me to concentrate harder on nature and not just take the big picture for granted. Trees are made of more than just branches and leaves. They consist of a mosaic of mosses, lichens, liverworts, bugs, and yes even Myxomycetes.

Myxomycetes are breath taking! One does not have to discover a new species to be awestruck by them. I believe that more people/students should be taught about nature's small wonders. In fact I believe the subject is so intriguing and important that I have excitedly begun to teach my parents about Myxomycetes: Melissa ends.

Observations and interactions between myxomycetes, mollusks, and nematodes

Student curiosity combined with being at the right place and right time led to serendipitous discoveries. Kenny Snell went collecting for myxomycetes with a flash light and fortuitously found an extensive area of stalked sporangia represented by immature soft, milky white, stages and mature, reddish brown stages with spores of the myxomycete *Stemonitis axifera* (Bull.) T. Macbr. The recent heavy rains several days before arriving at lodging quarters behind the Cades Cove Ranger Station in the GSMNP had created ideal moisture conditions for myxomycete development on the decayed tree trunks on ground sites. A *Quercus alba* L. log with exposed wood and bark sloughing off the sides was the source of the slugs *Philomycus carolinianus* (Bosc, 1802) and *P. flexuolaris* Rafinesque, 1820 (Mollusca: Gastropoda) that were crawling to the exposed surface areas of the wood from underneath the flaps of bark. Three successive nights on June 11, 12, and 13, 2001 these slugs selectively fed only on the developing immature sporangia, eating each sporangium from the top downward. Entire groups of the immature sporangia were eaten within two to three minutes. Details of the slug feeding activity were published and a color image showing the slug *P. carolinianus* eating the immature sporangium of *S. axifera* was selected for the front cover artwork of the journal *Mycologia* (Keller & Snell 2002).

Another student, Courtney Kilgore, had prepared moist chamber cultures of dead flower stalks and attached capsules of *Yucca smalliana* Fern. collected at Pertle Springs and also dead flower stalks and attached follicles of *Asclepias syriaca* L. collected from Taberville Prairie in Missouri. The white phaneroplasmodium of *Physarum cinereum* (Batsch) Pers. had developed on the substrata and migrated to the undersurface of the plastic Petri dish lid. The same

plasmodium was also present on the stems and capsules/follicles along with plasmodial tracks on the filter paper in the bottom of the Petri dish. While photographing the phaneroplasmodium, she observed nematodes on the underside of the Petri dish lid actively moving into and among the plasmodia. Hundreds of worms had migrated onto the lid around the plasmodium and were observed penetrating the plasmodial veins headfirst with their entire body inside the plasmodial veins and in some cases half inside and half outside. Interestingly, rotifers were also observed associated with the phaneroplasmodium (Kilgore and Keller 2008).

Simple experiments were designed to determine if the nematodes were actually feeding and ingesting the contents of the plasmodium and also the black spores of the mature sessile sporangia of *Physarum cinereum*. Four Petri dishes of 2% water agar were prepared and inoculated with the yellow sclerotia of *Physarum polycephalum* Schwein. Sterile old fashioned oat flakes were scattered on the agar surface and about 5 ml of sterile distilled water was added to create a thin film of water. Approximately 48 hours later the typical bright yellow phareroplasmodium and trailing network of veins had developed and covered the oat flakes and agar surface. The original *Yucca* and *Asclepias* plates were mist sprayed and 5 ml of excess water was added to the plasmodial cultures. The nematodes and rotifers apparently had encysted and the additional water had revived their motile stages because the agar plates were teeming with crawling nematodes and swimming rotifers.

Dr. Howard Ferris (pers. comm.) identified the nematodes as bacterial feeders in the genus *Panagrolaimus* and most similar to *P. rigidus* (Schneider, 1986) Thorne, 1937. The nematodes and rotifers were transparent and colorless so their internal contents could be observed. The head, feeding mouth parts, and internal parts of the nematodes and rotifers were observed with a compound microscope at 100 and 430 X magnification. Approximately four hours of careful observation over a four day period failed to confirm the presence of either nematodes or rotifers inside the plasmodial veins. Furthermore, the nematodes aggregated around the slime sheath along the plasmodial network of veins and appeared to feed on the surface areas presumably on concentrations of bacteria. Plasmodia developed into the typical multi-lobed spore cases with brown spores of *P. polycephalum*. No evidence was observed of nematodes and rotifers feeding on the dark brown myxomycete spores.

Other tree canopy cryptogams: fungi, lichens, mosses, liverworts, and ferns

Student climbers were given instructions to search for macrofungi along the bark surface from 3 to 30 m. Only three species of macrofungi were found at 4 to 6 m and only two corticioid species. Surprisingly, macrofungi were rarely observed in the tree canopy on the bark surface of healthy living trees (Keller 2004).

Tree canopy bark samples were examined for lichens and 195 taxa and of these, 84 apparently represent new lichen records for the GSMNP. *Phaeophyscia hispidula* (Ach.) Essl., a brown foliose lichen found in the canopy of *Juglans nigra* L. and *Liquidambar styraciflua* L., is primarily a northern species so that its occurrence in the canopy may represent a disjunct (Ciegler et al. 2003). *Gomphillus americanus* Esslinger is a rarely collected species confined to southeastern U.S.A. This abundant crustose lichen occurred at 15 m on *Fraxinus americana* and at 20 m on *L. styraciflua* intermixed with mosses. This lichen has

stalked, peltate, hyphophores 1-2 mm tall that have a conspicuous starburst appearance with a marginal fringe of sharp points. This collection represents a new record for the state of Tennessee and GSMNP. All lichen species recorded from the tree canopy in GSMNP also occur on ground sites (Keller 2004). Student climbers repeatedly observed that lichen growth and biomass increased near the top of the tree. This appears to be related more to sunlight as the canopy becomes more open at the top. Lichen observations at d.b.h. in densely shaded areas confirmed that lower levels of the trunk had less lichen cover than at higher levels of the tree trunk The foliose lichen growth form had the highest species richness based on bark samples gathered from the trunks of 4 different living tree species and 8 individual trees at 3 m increments to 36 m. The crustose lichen growth form had the second highest species richness and the fruticose growth form was only observed on one tree trunk but was more abundant on horizontal branches away from the trunk (Fanning et al. 2007). The first Lichen Bio-Quest was held in GSMNP June 19-20, 2004 resulted in 88 species, including seven new lichen records, and three new lichenicolous fungi. This paper has a noteworthy discussion about how to organize and lead field forays for the participants (Keller et al. 2007).

Epiphytic liverworts represented by 21 species were identified from bark samples gathered from 110 trunks of living trees at heights from 3.5 m to 30 m (Davison and Keller 2004). There were 37 moss species collected from the tree canopy. The light-loving moss, *Drummondia prorepens* (Hedw.) E. Britton, was collected frequently high in the tree canopy, yet it is only known from four ground sites in the GSMNP. All of the bryophyte species found in the tree canopy were also known from ground sites (Keller 2004).

The typically lithophilic fern, *Polypodium appalachianum* Haufler & Windham, was found growing as a canopy epiphyte 35 and 40 m above ground level on horizontal branches of a champion-size *Liriodendron tulipifera* in the GSMNP. Damon Lesmiester was the student climber who discovered this epiphytic fern growing as mature plants with immature and mature sori. Occurring along with this fern was an assemblage of terrestrial mosses including *Rhodobryum roseum*, an assortment of collembola (springtails), and a flightless proturan insect species (*Acerentulus confinis* (Berlese, 1908) only known from soil and litter. The distinctive features of this canopy habitat may effectively duplicate ecological conditions normally found only at ground level, establishing the opportunity for translocating an entire community and providing biologists with new insights on the origin of some epiphytes (Keller et al. 2003).

Research Experience for Teachers

The NSF-Research Experience for Teachers Program facilitates professional development of K–12 teachers on the cutting edge of science through partnerships between local school districts and universities (Keller 2005). Trish Smith, a Warrensburg Middle School seventh grade life science teacher, along with students and faculty from UCM, participated in a summer tree canopy biodiversity project in the GSMNP (Smith 2005, Smith and Keller 2004).

A website was created at http://warrensburg.k12.mo.us/iadventure/GSMNPiadventure/ where the field or Adventure Phase “Exploring Life in the Forest Canopy,” represented the first tier of the iAdventure website. This website enabled students and teachers to experience tree canopy research and learn about the All Taxa Biodiversity Inventory supported by Discover Life in America

(www.dlia.org). This was followed by the Laboratory Phase where students observed moist chamber cultures with wet bark that enabled students to observe a living miniature ecosystem composed of myxomycetes, fungi, lichens, mosses, liverworts, green algae, cyanobacterial algae, myxobacteria, tardigrades, insects, nematodes, and possibly other invertebrates. The students found several rare myxomycete species such as *Echinostelium arboreum* H.W. Keller & T.E. Brooks, known only from a few locations in the world.

The second tier of the website at http://warrensburg.k12.mo.us/iadventure/whatis.html was an iAdventure problem-solving activity. Students determined the direction and outcome of a content-rich storyline, using resources available on the Internet. This activity was designed to help students discover how to use and access data and information on the Internet and to solve problems and make choices. Students were expected to develop their own research questions and design their own experiment using the specimens and collected data. This subsequently led them to the Publication Phase, where they were expected to create poster presentations shared with parents and the school community. These classroom activities and website experiences encouraged secondary students to choose field biology as their future career (Smith & Keller 2004).

Questions, questions, and more questions

This tree canopy biodiversity research project has generated many more questions based on the data gathered. Clearly the bark pH differs between different species of living trees and only certain myxomycete species assemblages are associated with lower more acid pH, another group occurs in a middle pH range, and still another group occurs only near a neutral pH or slightly alkaline, and some fewer myxomycete species are generalists that occur from the lowest to the highest pH range. What physical and chemical properties of bark influence pH values? How important is the water absorption capacity of the bark? How does the age and size of the tree species influence species diversity, species richness, and myxomycete succession over time? Where should we look for new myxomycete species? What microorganisms are present on the bark of living trees that interact with the life cycle stages of myxomycetes? Why have so few corticolous myxomycetes been cultured from spore to spore especially species in the genus *Macbrideola* and *Cribraria*? What myxomycete species do we see most often together? What role does the percentage and distribution of epiphytic cover on the bark surface such as lichens, mosses, liverworts, green algae, cyanobacteria, have when associated with myxomycete species assemblages. Why are so few species of macrofungi found on the bark surface of living trees? How long does it take for myxomycetes to colonize the bark surface of living trees? There are still many areas of the Old World and New World Tropics that are unexplored but also the giant redwoods such as *Sequoia sempervirens* (Lamb. ex D. Don) Endl. in California that may live for more than 2,000 years and include the tallest trees on Earth. What myxomycete species are found on the bark surface of these “big trees” and other giant gymnosperms located in northwestern United States of America? The next generation of tree canopy biologists should consider answering these questions especially for myxomycetes and fungi. More research and exciting discoveries still await explorers in the tree canopy!

Acknowledgments

Our sincerest gratitude goes to more than 20 student climbers who participated in this project. Faculty and student colleagues in the Department of Biology and Earth Science at UCM who provided encouragement and logistical support that made this project possible, especially Joe Ely, who assisted in the field and climbed trees, served as the primary source of our statistical analysis, and contributed many long hours working with students on their research projects, reading thesis and journal papers.

The success of this tree canopy project depended on numerous individuals, including local residents in the GSMNP area who knew where to find a champion-sized tree or grapevines and professionals who offered their expertise in taxonomic identification and reviewing papers for publication. Keith Langdon and Paul Super from GSMNP gave assistance in obtaining collecting permits, maps, and directions to find big trees. Jeanie Hilten assisted with equipment needs, logistics, park directions, and lodging at GSMNP as part of Discover Life in America. Carolyn and Reid Franks generously offered their home and friendship to us on numerous occasions while we conducted research in GSMNP. David Taylor, botanist for the United States Forest Service at the DBNF in Kentucky, assisted in obtaining collecting permits, lodging facilities, and finding suitable tree climbing sites.

Many individuals accompanied the tree canopy team on expeditions to GSMNP and offered their expertise in a particular field, and participated as ground crew members. These individuals included: Alex Ciegler, Paul Davison, Uno Eliasson, Thomas Gaither, Diane Nelson, Kenneth Nelson, Jay Raveill, Trish and Stan Smith, and Ted Stampfer. Stephen Wilson assisted with canopy flight-intercept insect traps in GSMNP and Big Oak Tree State Park, served on graduate student thesis committees, edited theses, and reviewed papers for publication. Trish Smith involved seventh grade life science students at Warrensburg Middle School with the Research Experience for Teachers project by culturing myxomycetes in the classroom, developed an interactive website for students to participate in their own tree canopy adventure, and invited members of the UCM tree canopy team to visit the classroom to help identify organisms in moist chamber cultures. Uno Eliasson aided in collecting and identifying myxomycete specimens that were new records or had unusual characteristics, and he reviewed papers for publication. Karl and Jeanne Braun and Ted Stampfer reviewed papers for publication.

University of Central Missouri professional staff (Lisa Schmidt, Terry McNeeley) contributed many hours assisting with posters, power point presentations, web site construction, and Glenda Carmack volunteered her photographic skills to record our climbing activities at Pertle Springs, Missouri. Pamela Ganley, Coordinator for Evergreen Cemetery, provided photographs of *J. virginiana* (Figure 1) and information and details about the history of the cemetery. I (HWK) especially want to recognize the Association of Southeastern Biologists members and supporters for their friendship and camaraderie since the first meeting I attended held at Mobile, Alabama in 1972. Tim Atkinson from the Carolina Biological Supply Company has given invaluable advice over the years.

This project was financially supported in part by the National Science Foundation Division of Environmental Biology Awards #0079058 and #0343447, Discover Life in America Awards #2001-26, #2002-17, and #2004-6, National

Geographic Society Committee for Research and Exploration Award #7272-02, the Missouri Department of Natural Resources for the tree canopy research in Big Oak Tree State Park, Missouri, the United States Department of Education McNair Scholars Program, and the UCM Summer Undergraduate Research and Creative Projects Program.

Literature Cited

Ciegler, A., U.H. Eliasson, and H.W. Keller. 2003. Tree canopy lichens of the Great Smoky Mountains National Park. Evansia 20(4):114-131.

Counts, J., L. Henley, M. Skrabal, and K.L. Snell. 2000. Tree canopy biodiversity in the Great Smoky Mountains National Park. Inoculum 50:1-5.

Davison, P.G., and H.W. Keller. 2004. Vertical distribution of liverworts within the forest canopy in the Southern Appalachians: contributions to the All Taxa Biodiversity Inventory of the Great Smoky Mountains National Park. Evansia 21:79-87.

Everhart, S.E. 2007. Association and distribution of corticolous myxomycete species in the tree canopy of selected forests in Kentucky and Tennessee. (Master's thesis) University of Central Missouri. 75 p.

Everhart, S.E. and H.W. Keller. 2008. Life history strategies of corticolous myxomycetes: the life cycle, fruiting bodies, plasmodial types, and taxonomic orders. Fungal Diversity 29:1-16.

Everhart, S.E., H.W. Keller, and J.S. Ely. 2008. Influence of bark pH on the occurrence and distribution of tree canopy myxomycete species. Mycologia 100:191-204.

Fanning, E.F., J.S. Ely, H.T. Lumbsch , and H.W. Keller. 2007. Vertical distribution of lichen growth forms in tree canopiesof Great Smoky Mountains National Park. The Great Smoky Mountains National Park all taxa biodiversity inventory: a search for species in our own backyard. S.E. Naturalist 6:83–88.

Gray, W.D., and C.J. Alexopolous. 1968. Biology of the Myxomycetes. The Ronald Press Company, New York.

Ing, B. 1994. Tansley Review No. 62. The Phytosociology of Myxomycetes. New Phytol. 162:175–201.

Keller, H.W. 1979. Travis E. Brooks 1917-1976. Mycologia 71: 233-237.

Keller, H.W. 1996. Biosystematics of Myxomycetes: a futuristic view. Invited Plenary Address. In: C. Lado and J.C. Hernandez eds. Second International Congress on the Systematics and Ecology of Myxomycetes. Abstract Volume. 23–37. Madrid. Real Jardin Botánico, CSIC, Madrid, Spain.

Keller, H.W. 2004. Tree canopy biodiversity and student research experiences in Great Smoky Mountains National Park. Syst. Geogr. Pl. 74:47–65.

Keller, H.W. 2005. Undergraduate research field experiences: tree canopy biodiversity in Great Smoky Mountains National Park and Pertle Springs, Warrensburg, Missouri. CUR Quarterly 25(4):162–168.

Keller, H.W. and T.E. Brooks. 1973. Corticolous Myxomycetes I: two new species of *Didymium*. Mycologia. 65: 286-294.

Keller, H.W. and T.E. Brooks. 1977. Corticolous myxomycetes VII: contribution toward a monograph of *Licea*, five new species. Mycologia. 69: 667-684.

Keller, H.W. and K.L. Braun. 1999. Myxomycetes of Ohio: Their systematics, biology, and use in teaching. Ohio Biological Survey Bulletin New Series 13:1-182.

Keller, H.W., P. Davison, C. Haufler, and D.B. Lesmeister. 2003. *Polypodium appalachianum*: an unusual tree canopy epiphyte in the Great Smoky Mountains National Park. Am. Fern. J. 93(1):36-41.

Keller, H.W., J.S. Ely, H.T. Lumbsch , and S.B. Selva. 2007. Great Smoky Mountains National Park first lichen bio-quest. The Great Smoky Mountains National Park All Taxa Biodiversity Inventory: A Search for Species in Our Own Backyard. S.E. Naturalist 6:89–98.

Keller, H.W. and M. Skrabal. 2002. Discovery of a new obligate tree canopy myxomycete in the Great Smoky Mountains National Park. Newslett. MSA. Inoculum. 53(2):1-4.

Keller, H.W., M. Skrabal, U.H. Eliasson, and T.W. Gaither. 2004. Tree canopy biodiversity in the Great Smoky Mountains National Park: ecological and developmental observations of a new myxomycete species of *Diachea*. Mycologia 96: 537-547.

Keller, H.W. and K.L. Snell. 2002. Feeding activities of slugs on Myxomycetes and macrofungi. Mycologia 94:757-760.

Kilgore, C.M. and H.W. Keller. 2008. Interactions between myxomycete plasmodia and nematodes. Newslett. MSA. Inoculum 59 (1): 1-3.

Kilgore, C.M. 2008. Aerial reproductive structures on vascular plants as a microhabitat for myxomycetes. (Master's thesis) University of Central Missouri. 97 p.

Kilgore, CM, H.W. Keller, S.E. Everhart, A.R. Scarborough, K.S. Snell, M.S. Skrabal, C. Pottorff, and J.S. Ely. 2008. Tree canopy research and student experiences using the doubled rope climbing method. J. Bot. Res. Inst. Texas. 2(2): 1309-1336.

Martin, G.W. and C.J. Alexopoulos. 1969. The Myxomycetes. University of Iowa Press, Iowa City.

Moore, B. 1996. The Lochsa story: land ethics in the Bitterroot Mountains. Mountain Press Publishing Company. Missoula, Montana.

Novozhilov, Yu.K., M. Schnittler, I.V. Zemlianskaia, and A.F. Konstantin. 2000. Biodiversity of plasmodial slime moulds (Myxogastria): Measurement and interpretation. Protistology, 1: 161–178.

Novozhilov Yu.K., I.V. Zemlianskaia, M. Schnittler, and S.L. Stephenson. 2006. Myxomycete diversity and ecology in the arid regions of the Lower Volga River Basin (Russia). Fungal Diversity23:193-241.

Schnittler M. 2001. Ecology of myxomycetes of a winter-cold desert in western Kazakhstan. Mycologia 93:653-669.

Schnittler M., S.L. Stephenson, and Y.K. Novozhilov. 2000. Ecology and world distribution of *Barbeyella minutissima* (Myxomycetes). Mycological Research 104: 1518-1523.

Smith, P.A. 2005. National Science Foundation Funds Research Experience for Teachers. Southeastern Biology. 52 (1): 43-45.

Smith, P.A. and H.W. Keller. 2004. National Science Foundation Research Experience for Teachers (RET). Newslett. MSA. Inoculum. 55(6):1-5.

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

Snell, K.L. 2002. Vertical distribution and assemblages of corticolous myxomycetes on five tree species in the Great Smoky Mountains National Park. (Masters thesis) Central Missouri State University. 47 p.

Snell, K.L. and H.W. Keller. 2003. Vertical distribution and assemblages of corticolous myxomycetes on five tree species in the Great Smoky Mountains National Park. Mycologia 95:565-576.

Snell, K.L., H.W. Keller, and U.H. Eliasson. 2003. Tree canopy myxomycetes and new records from ground sites in the Great Smoky Mountains National Park. Castanea. 68(2):97-108.

Unterseher, M., M. Schnittler, C. Dormann, and A. Sickert. 2008. Application of species richness estimators for the assessment of fungal diversity. FEMS Microbiol. Lett. 282:205–213. ☙