Influence of bark pH on the occurrence and distribution of tree canopy myxomycete species

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Abstract: This study compares the occurrence and distribution of myxomycete species in the canopy of living trees and neighboring grapevines. Corticolous myxomycetes of three temperate forests in southeastern USA were studied on six tree species (30 trees) and grapevines (30 vines) to determine distribution and occurrence of myxomycete species relating to geographic location, host species, and bark pH. The double-rope climbing technique was used to access the canopy and sample bark up to 16.5 m. Bark samples were examined in 580 moist chamber cultures and 44 myxomycete species were identified representing 21 genera, averaging 3.0 ± 2.1 species per sample site. Jaccard's coefficient determined community similarity between 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*. Vertical variation in species richness was significantly different only for *Platanus occidentalis* and might be attributable to flaking of bark with increasing height in the canopy. *Tsuga canadensis* and neighboring grapevines had greatest community similarity. *Cribraria violacea* was observed on all tree and grapevine species except *T. canadensis* and neighboring grapevines. Occurrence and species assemblages of myxomycetes were associated with bark pH, not geographic location. Bark of *V. aestivalis* (pH 4.5) was more acidic than neighboring *T. canadensis* (pH 4.1), compared to grapevines of the same species neighboring other tree species. Results indicated that most species are not regionally restricted, and although some myxomycetes are associated with a certain pH range, others develop on any substratum. Future research protocols for corticolous myxomycetes should emphasize sampling adequate amounts of substrata in a local region from different host species that have a wide range of bark pH, ensuring a representative sample of species for an entire region.

INTRODUCTION

Myxomycetes are Protista characterized by a spore that gives rise to biflagellate swarm cells or myxamoebae which, after syngamy, form a multinucleate plasmodium that eventually gives rise to one or many fruiting bodies. Myxomycetes have been found throughout the world, with the highest diversity documented in temperate forests (Spiegel et al 2004). Within temperate forests some species inhabit only the forest floor on decayed wood, other species are found on decaying leaf litter and still others only occur on the bark of living trees and vines (Keller and Braun 1999).

Myxomycetes that complete their life cycle from spore to fruiting body formation on the bark of living trees and vines are termed corticolous myxomycetes (Keller and Brooks 1973). Although certain species, such as *Fuligo septica* (L.) F.H. Wigg. and *Lycogala flavofuscum* (Ehrenb.) Rostaf., have been found fruiting up to 2 m on the bark of living trees, they typically complete their life cycle on decaying leaves and logs on the forest floor. For this reason collecting true corticolous myxomycetes involves sampling bark above 3 m, here defined as the tree canopy.

Corticolous myxomycetes in the tree canopy were studied first by Snell and Keller (2003). They found a few common species among all tree species, and different species assemblages of myxomycetes were associated with different tree species and bark pH but showed no difference with respect to height in the canopy. On individual trees, distribution of myxomycetes on each tree was uneven and patchy, and the community of myxomycete species varied considerably from tree to tree. Using Sorensen's community coefficient, Snell and Keller (2003) noted that myxomycete communities on trees of the same species had low similarities with one another, while trees of different species had some of the highest similarities. These results are counterintuitive and might indicate regional occurrence of myxomycete species. Unfortunately comparisons between trees from the same location were not made. Contrary to this hypothesis other studies suggest that species are

not regionally restricted because the spores are wind-dispersed over large distances (Stephenson 1989, Tesmer and Schnittler 2007).

The purpose of this study was to determine whether the uneven distribution of myxomycete species in the tree canopy is regional or restricted to individual trees. Trees and neighboring grapevines were sampled from different locations to examine distribution and occurrence of myxomycete species in relation to bark pH, height in the canopy, tree or grapevine species, and geographic location. Comparisons along a vertical transect between different host species can be made by sampling grapevines and their neighboring tree. Furthermore grapevines are restricted to the same location, usually growing within 2 m of the host tree. Thus it was expected that the proximity of hosts will result in a more similar assemblage of myxomycete species. Comparisons were made within and between geographic locations to test the hypothesis that myxomycete species are randomly distributed. The null hypothesis states there is no difference in the assemblages and distribution of myxomycete species among host tree species and neighboring grapevine species. The goal of the study was to further our understanding of the ecology of corticolous myxomycete species and determine factors that influence their occurrence and distribution.

MATERIALS AND METHODS

Study areas.—Three discontinuous temperate forests in southeastern USA were selected for this study because of favorable climatic conditions, variety of habitats, and high diversity of tree and vine species (Stupka 1964, Jones 2005).

Great Smoky Mountains National Park (GSMNP) covers an area approximately 210 545 ha (est. 1930) and is located on the North Carolina and Tennessee border $35°28'$–$35°47'$N, at the southernmost range of the Appalachian Mountains. The average yearly rainfall is 140–216 cm (Shanks 1954). Approximately 40% of the GSMNP is virgin, old growth forest (Stupka 1964). Typical dominant tree species within most of GSMNP are yellow-poplar (*Liriodendron tulipifera* L.), eastern hemlock (*Tsuga canadensis* (L.) Carr.), and a variety of oak species (*Quercus* spp.), underlain by thick understory cover of rhododendron (*Rhododendron* spp.) (Stupka 1964). Grapevines also are recorded in the area, with a record size grapevine, thought to be *Vitis vulpina* L., 48 cm diam at breast height (DBH, 1.5 m) recorded near Dunns Creek, GSMNP, in 1935 (Stupka 1964). This is thought to be only one of six species of *Vitis* that are known to occur in GSMNP (Stupka 1964).

Daniel Boone National Forest (DBNF) contains both public and private land in 21 eastern Kentucky counties from Rowan in the northwest ($36°36'$N) to McCreary in the south and to the Tennessee border ($38°24'$N), spanning 225 km on the eastern edge of the Cumberland Plateau. Established as the Cumberland National Forest in 1937, the name was changed to Daniel Boone National

Forest in 1965 and today covers an extensive, rugged area of more than 258 440 ha, including the Clifty Wilderness Area to the east (5000 ha), Beaver Creek Wilderness Area (2000 ha), and Red River Gorge Geological Area (10 500 ha) (Collins 1975). In contrast to GSMNP the climate in Kentucky is drier, with an annual precipitation of 104–135 cm that varies from north to south (Martin et al 1993). DBNF is mostly forested and characterized by sandstone cliffs, bluffs, caves, arches and natural bridges. The forest type is within the mixed-mesophytic region of the eastern deciduous forest with tree species such as yellow-poplar (*Liriodendron*), hickory (*Carya* spp.), maple (*Acer* spp.), and eastern hemlock (*Tsuga canadensis*). Protected within DBNF are more than 750 flowering plants species and 170 moss species (Hopkins 1996).

Berea College Forest (BCF), Kentucky, is privately owned and managed by Berea College. The forest is to the east of DBNF, near Berea, Kentucky, also on the edge of the Cumberland Plateau ($37°35'$N; $84°15'$W and $37°30'$N; $84°15'$W and $32°30'$N; $84°16'$W and $32°30'$N; $84°12'$W). The physical features and vegetation are similar to those of DBNF. Most of the 3200 ha was acquired 1898–1960, before which it was in poor condition due to heavy logging, farming and grazing. The area was reforested by the college and now is managed for recreation, water, wildlife and wood (Perry 2000).

Field methods.—Field expeditions were conducted during summer 2006 in GSMNP from 1–13 and 25–29 Jun, DBNF from 1–9 Aug and BCF from 15–23 Jun and 18–30 Jul. Myxomycetes were collected from ground sites (decaying logs and leaf litter) and bark from the tree canopy was used for moist chamber culture. The double-rope climbing technique was used to access the tree canopy.

Suitable climbing trees supporting grapevines were difficult to locate, and random selection of trees with grapevines was not possible. Methods for locating trees with grapevines included trail guides, vegetation maps, and consultations with local residents, park and state officials and hikers. The selection criteria were trees with a minimum of 60 cm trunk diam at breast height (DBH=1.5 m) and neighboring grapevines with a minimum of 4 cm DBH. These criteria excluded many tree-vine combinations that allowed bark sampling higher than 16.5 m. For example many suitable climbing trees had grapevines that were too small or many grapevines were large enough but were either not on trees suitable for climbing or were purposely cut at the base and dead. Grapevines in the DBNF often were dead, especially in the Red Bird District in part because ''grapevine pullers'' are permitted to remove vines from trees to handcraft wreaths and chairs that represent a source of income for residents in this area. This tree-grapevine criterion made attempts at random selection impractical.

The double-rope climbing technique used to access the canopy is described in detail by Jepson (2000) and Everhart (2007). Suitable climbing trees were free of dead branches, poison ivy and thorns. A large slingshot was used to launch a pellet-weighted throw bag with a lightweight, slick line over the desired limb. The slick line was attached to the climbing rope and pulled over the limb. Climbers wore an arborist's saddle and helmet, and ''tied in'' by anchoring one end to the saddle and tying a friction knot, using a split-tail, onto the running end. The double-rope climbing technique was preferred over other tree climbing techniques because all equipment was easily carried in

backpacks, did no damage to trees and let the climber advance the rope to higher branches.

Bark sampling.—Only trees and grapevines that allowed sampling in a vertical transect, every 3.3 m, 3.3–16.5 m were sampled. Bark was collected by evenly prying off samples from areas within reach, avoiding damage to living tissue and half filling a paper bag (ca. 1000 cm^3). When bark was wet, due to high humidity or rainfall, it was air-dried on newspapers at least 24 h before repackaging. Every bag was labeled with the identifying number, tree or vine and height of bark sample.

Tree height was measured with a reel-bound altitude tape attached to the climbing saddle. Each tree was given a unique identifying number that referred to both the tree and grapevine. A small tag with the identification number was attached to the tree approximately 6–10 m high on the opposite side from the trail or road, out of direct line of sight. Ground crew members were responsible for recording a data sheet of information with tree identification number, tree species, Universal Transverse Mercator coordinates, elevation, tree and grapevine DBH, height of the tree, and general observations such as weather conditions and proximity to geographic features (streams, rocky outcroppings and trails).

Leaf voucher specimens were collected for both the tree and grapevine. Grapevines often were difficult to reach due to their growth habit, leafing out above the outer canopy of treetops. When the leaves from the outer canopy (sun leaves) were not accessible, leaves from sucker shoots were collected. Tree species identifications were verified by Jay A. Raveill, University of Central Missouri, and grapevine identifications were verified by the current expert on Vitaceae, Jean M. Gerrath, University of Northern Iowa. Identification of grapevine species was more difficult due to intergradations of taxonomic characters and paucity of characters based on dried, herbarium specimens. Due to these difficulties a key to grapevine species was designed for identification of dried voucher specimens of species known to occur in GSMNP, DBNF and BCF (Everhart 2007).

Laboratory methods.—Moist chamber cultures were prepared for 30 trees and 30 neighboring grapevines, representing six tree and two grapevine species. Ten moist chamber cultures were prepared for each tree and each grapevine. We established two moist chamber cultures for each bark sample that were collectively referred to as a ''sample site'' (Snell and Keller 2003). Moist chamber cultures from individual trees and neighboring grapevines were prepared at the same time. Bark was selected randomly from bags by mixing the pieces within the bag and then selecting pieces that touched the hand first. Each moist chamber culture was an oversize, sterile, plastic Petri dish (150 × 25 mm) that was large enough to enclose thick bark pieces. Tree identification number, tree (T) or vine (V), height and replicate number were labeled on the lid and sides of each dish.

Bark was placed inside Petri dishes and arranged in a single layer on pH neutral, sterile P8 grade circular filter paper (150 mm diam). Bark was wetted with 35 mL of sterile, deionized water adjusted with a combination of KOH and HCl to pH 7.0. An Orion model 610 flat probe meter measured pH. Water was poured directly onto bark and then tilted back and forth to prevent uneven pooling. Moist chambers were incubated in ambient light and room temperature (23–25 C). After 24 h pH was measured in three random places on the filter paper, close to the bark or under bark pieces. An additional pH measurement was taken for water before decantation, with the Petri dish tilted 45 degrees.

Bark was scanned for presence of myxomycetes with a dissecting stereomicroscope at 70× magnification on day 4, 8, 16 and 32. When water condensed on the lid of the Petri dish the lid was removed and replaced with a dry one, to increase visibility while scanning. Each moist chamber culture was scanned systematically, starting at the top and moving from side to side. A pin was placed near immature myxomycete fruiting bodies; if mature the myxomycete was identified with the key to species by Martin and Alexopoulos (1969). Water-mount slides of myxomycetes were made and examined under oil magnification for identification of the peridium, capillitium, and spore characteristics. Spores were measured with a calibrated ocular micrometer. Permanent slides were made by mounting the fruiting body in clear lactophenol, sealed with a resiniferous slide-ringing compound and on the frosted end labeled in pencil and covered with clear tape for protection. Rare or possible new species were removed from moist chamber culture before day 32 and before mold growth.

Voucher specimens of myxomycetes were made after all data were collected, with separate voucher boxes for each species from an individual tree or grapevine. Bark with myxomycete fruiting bodies was removed and glued into the bottom of a standard collection box, 4.5 × 10.5 × 2 cm (Keller and Braun 1999). Labels with species name, collection location, habitat, UTM coordinates, elevation, bark collection date, wet date, harvest date, collector's name, and accession number were affixed to box tops. Myxomycete voucher specimens were sent to the United States National Fungus Collections (BPI), Beltsville, Maryland.

Data analysis.—Jaccard's community coefficient was used for pairwise comparison of myxomycete communities on individuals of each tree species and grapevine species that were segregated by neighboring tree species. This distance measure reflects the proportion, in city-block space, of species common between two communities. Jaccard's coefficient equals w/(A + B − w), where w is the number of species common to both communities A and B and the sum of species abundances in both communities minus species common to both is the denominator. PC-ORD statistical software calculated a distance matrix which, when two items differ, yields a positive distance. Values in the distance matrix were used to express similarity by subtracting from one and then multiplying by 100. Although Sorenson's community coefficient has been used for similar studies (Snell and Keller 2003) Jaccard's coefficient was selected because the species data are binary, present or absent (Legendre and Legendre 1998). In addition Jaccard's coefficient also was chosen because of its robust nature with respect to communities with few common species, such as the corticolous myxomycetes, and intuitive nature of the expression when converted to percent similarity between communities (McCune and Grace 2002). Grapevines of the same species first were compared in pairwise fashion by their neighboring tree species and, if showing no difference, five grapevines of each species were selected randomly for comparison between grapevine species and tree species. Mean ± SE bars were compared to determine differences.

Data were tested for normality with Anderson-Darling test ($\alpha = 0.05$) and homogeneity of variance using Levine's test ($\alpha = 0.05$). All data failed to meet the assumptions of analysis of variance, lacking normal distribution of samples and homogeneity of variance and therefore were compared for significant differences using standard error. Graphical representations of data were made with Sigma-Plot version 9.01 and compared with the use of standard error bars (Zar 1999). All descriptive statistics were calculated with PC-ORD version 4.27, and comparisons were made with Minitab Release 14.13 and SPSS 12.0.1 statistical software.

Analysis of vertical variation first was restricted to trees and grapevines of the same species. Myxomycete species richness at each height was compared to species richness of all other heights of the same tree or grapevine species. Comparison of mean ± SE for species richness was used to determine differences in vertical variation.

Mean bark pH was determined by converting measurements to hydrogen ion concentration (linear scale), finding the mean and then converting back to pH values (logarithmic scale). Standard error (SE) for pH likewise was calculated in hydrogen ion concentration but cannot be converted directly back to the pH scale. Therefore standard error in hydrogen ion concentration was determined by adding it to the mean hydrogen ion concentration and then converting to pH. This addition gave the lower end of the SE of pH; the upper end was determined with the same operation but subtracting SE in hydrogen ion concentration from the mean hydrogen ion concentration and then converting to pH (Guare 1991). The calculated upper and lower SE of hydrogen ion concentrations are used to determine the range of error, which is divided by two to find pH ± SE. Uncertainty in SE of mean pH was determined as the first place value where variation between upper and lower SE begins (Guare 1991). Both of these statistics are routinely miscalculated by not converting pH values to hydrogen ion concentration. Due to the lack of independence, nonnormality, and lack of homogeneity of values on the pH scale, the previously calculated means and SE values are used to determine significance, where nonoverlapping SE ranges indicate significant difference.

Nonmetric multidimensional scaling (NMS), multiresponse permutation procedure (MRPP) and indicator species analysis (ISA) were performed with PC-ORD statistical software to determine relationships between myxomycete species and environmental parameters. These nonparametric analyses were chosen because the data were nonnormal, lacked homogeneity of variance and were used to define groups, associations and myxomycete species assemblages. An alpha or significance level of 0.05 for the ISA and a Bonferroni correction for MRPP (α adjusted to 0.01) were used to determine significance (McCune and Grace 2002).

Nonmetric multidimensional scaling (NMS) was used to explore the possible relationships of myxomycete species and sample sites among all measured environmental factors for each host tree and grapevine species. Each NMS was performed with a random starting configuration, run using Sørensen's distance measure and tested for significance with the Monte-Carlo test. The number of axes was determined by stress values, where a value of 10–20 is typical for ecological data and a value closer to 10 than 20 is acceptable; lower stress is better. Joint biplots of samples and species results show the grouping of sites and either environmental parameters or myxomycete species as vectors related to the groups, where strength of the association to groups corresponds to length of the vector line and numerically represented by Kendall's tau. Axes shown in joint biplots were selected for the best grouping of sample units and strong associations with measured environmental parameters (McCune and Grace 2002).

Multiresponse permutation procedure is similar to multivariate analysis of variance but avoids the distributional assumptions of data normality and homogeneity of variance. MRPP was used to test the hypothesis of no difference between two or more groups or entities and used Euclidian distance because it is used most commonly in early literature on the analysis. Results are interpreted from three values: delta (δ), T and A. Delta is the weighted mean of within group distance that describes how groups are defined and also gives the sizes of the groups. The test statistic T describes the separation between groups, where a more negative T indicates greater separation. Finally A is the chance-corrected within-group agreement or homogeneity, compared to the random expectation. Values for A are commonly less than 0.1 in community ecology studies, even when the observed delta is significant. Values of A of 0.3–1 (where A=1 indicates all items are identical within groups) are considered high for ecological data and therefore indicate a nonrandom difference in grouping (McCune and Grace 2002).

Results of indicator species analysis determine the constancy or faithfulness of species to a group by weighted averaging, which is the product of the relative abundance multiplied by relative frequency, times 100. In this way each species is given an importance value (IV) and indicator species for each group or cluster are those with the highest absolute value of IV. Significance is tested with a nonparametric procedure involving the Monte-Carlo permutation procedure (McCune and Grace 2002, Legendre and Legendre 1998, Dufrêne and Legendre 1997). Species with significant, nonrandom association with trees and grapevines, as determined by NMS, MRPP and ISA were used to determine myxomycete species assemblages associated with each tree and grapevine species.

RESULTS

Bark was selected from six tree species *Acer saccharum* Marsh. (Aceraceae), *Fraxinus americana* L. (Oleaceae), *Liquidambar styraciflua* L. (Hamamelidaceae), *Liriodendron tulipifera* L. (Magnoliaceae), *Platanus occidentalis* L. (Platanaceae), and *Tsuga canadensis* (L.) Carriere (Pinaceae). These trees were selected because they are in different families, have a variety of bark physical characteristics as described by Everhart (2007), and meet the minimum sample size of five individuals per species. (Each site is represented in TABLE I.) Bark from five trees of each species and grapevine species, *Vitis aestivalis* Michx. or *V. vulpina* L., was used to prepare individual moist chamber cultures.

Bark pH was measured 1841 times, produced nonnormal distributions and lacked homogeneity of

TABLE I. Number of trees and neighboring grapevines by study area with mean bark pH $\pm$ SE in order of increasing alkalinity

Tree / vine species	GSMNP	DBNF	BCF	Total	mean pH $\pm$ SE
Tsuga canadensis	5			5	4.1 $\pm$ 0.08[a]
V. aestivalis (*T. cana*) *	5			5	4.5 $\pm$ 0.05[b]
V. aestivalis (*F. amer*) *			3	3	4.8 $\pm$ 0.10[c]
Liriodendron tulipifera	4	1		5	5.0 $\pm$ 0.03[d]
Vitis aestivalis (*A. sacc*) *	5			5	5.1 $\pm$ 0.04[d]
Platanus occidentalis		5		5	5.1 $\pm$ 0.04[de]
V. aestivalis (*L. tuli*) *	4	1		5	5.2 $\pm$ 0.03[e]
V. aestivalis (*L. styr*) *	5			5	5.4 $\pm$ 0.10[f]
Acer saccharum	5			5	5.5 $\pm$ 0.05[f]
V. vulpina (*F. amer*) *		2		2	5.5 $\pm$ 0.08[f]
V. vulpina (*P. occi*) *		5		5	5.7 $\pm$ 0.03[g]
Liquidambar styraciflua	5			5	5.8 $\pm$ 0.04[h]
Fraxinus americana		2	3	5	6.3 $\pm$ 0.07[i]

Abbreviations: GSMNP = Great Smoky Mountains National Park, DBNF = Daniel Boone National Forest, BCF = Berea College Forest.

Superscript letters indicate significant differences in pairwise comparisons usingstandard error.

* Grapevine species with associated tree species given in parentheses.

variance when categorized by tree and grapevine species. Significant differences were determined by nonoverlapping standard error for the means. No significant difference was measured in pH near bark versus that when measured in the Petri dish tilted 45 degrees for each tree and vine species. Therefore pH measurements for each height on each tree and grapevine were combined. Bark pH was first compared independently by height among each individual tree and grapevine, then among trees and grapevines of the same species and finally pooled by species and compared. (The mean and standard error for each bark pH by tree and grapevine species is displayed in TABLE I, listed in order of ascending bark pH.)

Significant differences were found with respect to bark pH based on species of tree or grapevine, and with respect to *V. aestivalis* neighboring different tree species. *Tsuga canadensis* (4.1 $\pm$ 0.06) had a significantly different pH than all others, as did the neighboring grapevine, *V. aestivalis* (4.5 $\pm$ 0.05). No signifigant difference in mean pH was found between *L. tulipifera, V. aestivalis* neighboring *A. saccharum,* and *P. occidentalis,* or between *P. occidentalis* and *V. aestivalis* neighboring *L. tulipifera,* or between *V. aestivalis* neighboring *L. styraciflua, A. saccharum,* and *V. vulpina* neighboring *Fraxinus americana.* All others showed significant differences based on mean bark pH.

The bark of 30 individual trees and 30 grapevines was sampled, and 580 moist chamber cultures yielded a total of 44 myxomycete species, representing 21

genera, with an additional two taxa identified only to genus. Each sample height on a tree or grapevine is referred to as a sample site, from which we recorded an average of 2.9 $\pm$ 0.14 myxomycete species per site (Snell and Keller 2003). There was one new species record for GSMNP, *Perichaena pedata* (A. & G. Lister) G. Lister. In Kentucky there were 26 new records (10 only found in DBNF and two found in BCF), two of which were recorded as part of a master's thesis (Rothwell 1951) but not published records, such as the published abstract by Ford (1978) and two published papers by Branson (1988, 1990). (A full list of species found in each study site is listed as an appendix in Everhart 2007.)

Species richness and frequency.—Collective myxomycete species lists for each tree show that *Fraxinus americana* and *Liriodendron tulipifera* have the highest species richness, with 20 species each (TABLE III). Myxomycete species abundance was highest for *Tsuga canadensis* (220 species), *F. americana* (132 species) and *L. tulipifera* (118 species). Although *Vitis aestivalis* and *V. vulpina* had higher species richness (38 and 25 species) and higher species abundance (785 and 256 species), they also had larger sample sizes, with 222 and 68 moist chamber cultures respectively. Nevertheless relative abundance of myxomycete species per individual tree or grapevine showed that *T. canadensis* (37.0 $\pm$ 4.7) had the greatest, followed by *V. vulpina* (31.7 $\pm$ 4.8) and *V. aestivalis* (27.6 $\pm$ 2.0). Similarly the number of species per site was highest

TABLE II. Myxomycete species occurrence on trees and neighboring grapevines

Species	Study area	No. hosts	No. sites	Tree / vine species	% co-occurrence
Echinostelium minutum	B, D, G	39	125	A, F, L, P, T, a, v	46
*Arcyria cinerea**	B, D, G	37	87	A, F, L, P, T, a, v	42
Perichaena chrysosperma	B, D, G	33	77	A, F, L, P, S, a, v	52
*Cribraria violacea**	B, D, G	29	80	A, F, L, P, S, a, v	58
Cribraria confusa	G	29	20	A, T, a	14
*Comatricha ellae**	B, D, G	22	62	A, F, L, P, S, T, a, v	38
*Calomyxa metallica**	B, D, G	19	35	A, F, L, S, a, v	19
Physarum nutans	B, D, G	19	34	A, F, L, S, T, a, v	33
*Clastoderma debaryanum**	B, D, G	15	26	T, a, v	15
*Clastoderma pachypus**	B, D, G	14	30	F, L, T, a, v	17
Lamproderma biasperosporum	B, D, G	14	25	A, F, L, P, S, T, a, v	15
*Echinostelium coelocephalum**	D, G	13	28	P, a, v	9
Physarum crateriforme	B, D, G	13	27	F, P, a, v	44
Licea minima	G	12	5	L, S, a	0
Dianema sp.*	B, D, G	11	22	A, F, L, a, v	33
*Licea operculata**	B, D, G	11	22	L, T, a, v	33
*Diderma chondrioderma**	B, D, G	11	16	A, F, L, a, v	10
Macbrideola cornea	B, G	10	19	F, L, S, a	50
*Enerthenema papillatum**	B, D, G	9	14	A, F, T, a	13
Macbrideola decapillata	D, G	9	14	A, F, L, P, S, v	25
Licea parasitica	G	8	35	L, T, a	57
Perichaena depressa	D, G	8	14	a, v	–
Cribraria minutissima	G	8	4	T	–
Licea kleistobolus	G	5	7	L, a	67
Licea pedicellata	B, D, G	4	12	F, T, a, v	14
Licea marginata	B, D, G	4	8	L, a, v	0
Physarum sp. (silver peridium)	D, G	4	5	A, S, L, v	0
*Comatricha laxa**	D, G	4	4	A, L, S, a	0
*Licea biforis**	B, G	4	4	a, v	–
Minakatella longifila	D, G	3	4	a, v	–
Badhamia rugulosa	G	2	4	a	–
*Stemonitis axifera**	D, G	2	3	a, v	–
*Macbrideola scintillans**	G	2	2	A, F	–
*Physarum auriscalpium**	B	2	2	a	–
Physarum melleum	G	1	2	a	–
Badhamia sp. nov. †	G	1	1	F	–
Comatricha acanthodes	G	1	1	T	–
Diderma effusum	G	1	1	a	–
Hemitrichia sp.	G	1	1	L	–
Lycogala epidendrum	B	1	1	a	–
Perichaena pedata†	G	1	1	S	–
Physarum galbeum	G	1	1	a	–
Physarum oblatum	G	1	1	a	–
Physarum pusillum	G	1	1	a	–
*Physarum synsporum**	D	1	1	v	–
*Stemonitis curiosa**	D	1	1	v	–
Trichia contorta	G	1	1	S	–
Wilkomlangea reticulata	G	1	1	a	–

Species abbreviations: A = *Acer saccharum*, F = *Fraxinus americana*, S = *Liquidambar styraciflua*, L = *Liriodendron tulipifera*, P = *Platanus occidentalis*, T = *Tsuga canadensis*, a = *Vitis aestivalis*, v = *V. vulpina*.

Study area abbreviations: B = BCF, D = DBNF, G = GSMNP.

* New Kentucky state record for the species (Ford 1978, Branson 1988; 1990).

** Recorded for Kentucky only in an unpublished thesis (Rothwell 1951).

† New record for the species in Great Smoky Mountains National Park.

TABLE III.   Myxomycete frequency and species richness by tree and neighboring grapevine species

Host species	Total species	Mean no. of species ± SE	No. moist chambers	No. records	Mean abundance per sample site
Acer saccharum	17	8.4 ± 3.5	46	38	1.9 ± 0.5
Fraxninus americana	20	24.4 ± 3.5	46	132	5.3 ± 0.5
Liquidambar styraciflua	10	13.4 ± 3.3	50	81	2.7 ± 0.4
Liriodendron tulipifera	20	21.8 ± 8.1	50	118	4.4 ± 0.8
Platanus occidentalis	10	8.8 ± 1.6	48	59	1.8 ± 0.4
Tsuga canadensis	17	37.0 ± 4.7	50	220	7.4 ± 0.7
Vitis aestivalis	38	27.6 ± 2.0	222	785	5.7 ± 0.3
V. vulpina	25	31.7 ± 4.8	68	256	6.5 ± 0.7

for *T. canadensis* (7.4 ± 0.7), followed by *V. vulpina* (6.5 ± 0.7) and *V. aestivalis* (5.7 ± 0.3). Lowest species richness was *Liquidambar styraciflua* (10) and *Platanus occidentalis* (10), lowest number of myxomycete observations was *Acer saccharum* (38) and *P. occidentalis* (59), while lowest relative abundance of myxomycete species was *A. saccharum* (8.4 ± 2.6) and lowest species per site was *P. occidentalis* (1.8 ± 0.4). No vertical variation in species richness was measured for any tree or grapevine species except *P. occidentalis.*

Community analysis.—Comparing Jaccard's community coefficient calculated for each individual host species resulted in a total of 3721 comparisons that were converted to percent similarity and from which the mean and SE for each host species, arranged in order of increasing alkalinity, is shown (TABLE IV) as a square matrix. Species with *N* size equal to 2 resulted in only one calculation of percent similarity and therefore did not yield a mean and SE when making same-host species comparisons and because significant difference could not be determined in those cases the columns were deleted for simplicity.

Significant difference between communities associated with individuals of each host species was determined by nonoverlapping SE of the mean. The lowest similarity (3%) between myxomycete communities on different host species was found on *L. styraciflua* compared to *V. aestivalis* neighboring *T. canadensis.* Even less similarity (1%) was found when comparing myxomycete communities on individual trees of *A. saccharum,* however two of these trees resulted in no myxomycetes and therefore reduced the *N* size to three trees. Among the trees and grapevines that had five individuals, *L. tulipifera* (11%) had the lowest similarity with one another, while *V. vulpina* neighboring *P. occidentalis* (41%) had the highest similarity with one another.

Comparison of myxomycete species assemblages on trees and neighboring grapevines showed that the combination of *T. canadensis* and its neighboring grapevines, *V. aestivalis,* had the highest similarity (26%), *A. saccharum* and its neighboring grapevines, *V. aestivalis,* had the lowest similarity (10%), and all other combinations were 15–20% similarity. *Tsuga canadensis, V. aestivalis* neighboring *T. canadensis* and *A. saccharum* had the greatest number of

TABLE IV.   Comparison of myxomycetes on each host species and neighboring grapevine using Jaccard's coefficient of similarity

| Host	N	Tc	Va-Tc	Lt	Va-As	Po	Va-Lt	Va-Ls	As	Vv-Po	Ls	Fa
T. canadensis	5	33 ± 3										
V. aestivalis (*T. cana*)	5	26 ± 2	39 ± 3									
V. aestivalis (*F. amer*)	2	9 ± 2	15 ± 3									
L. tulipifera	5	17 ± 2	20 ± 3	11 ± 3								
V. aestivalis (*A. sach*)	5	15 ± 1	24 ± 2	16 ± 2	26 ± 4							
P. occidentalis	5	8 ± 1	7 ± 1	7 ± 1	12 ± 2	23 ± 4						
V. aestivalis (*L. tuli*)	5	16 ± 2	21 ± 2	15 ± 3	22 ± 2	12 ± 2	17 ± 4					
V. aestivalis (*L. styr*)	5	10 ± 1	14 ± 1	11 ± 2	26 ± 3	20 ± 2	18 ± 2	28 ± 5				
A. saccharum	3	10 ± 2	10 ± 2	8 ± 2	10 ± 2	15 ± 3	8 ± 2	13 ± 3	1 ± 1			
V. vulpina (*F. amer*)	2	11 ± 1	19 ± 2	10 ± 2	19 ± 3	16 ± 1	21 ± 3	20 ± 2	12 ± 2			
V. vulpina (*P. occi*)	5	9 ± 1	10 ± 1	7 ± 1	9 ± 3	20 ± 2	18 ± 2	22 ± 2	13 ± 2	41 ± 4		
L. styraciflua	5	6 ± 1	3 ± 1	4 ± 1	5 ± 1	22 ± 3	6 ± 1	15 ± 2	12 ± 2	24 ± 2	29 ± 3	
F. americana	5	4 ± 1	7 ± 1	5 ± 1	6 ± 1	12 ± 1	7 ± 1	12 ± 2	10 ± 2	15 ± 2	17 ± 2	17 ± 3
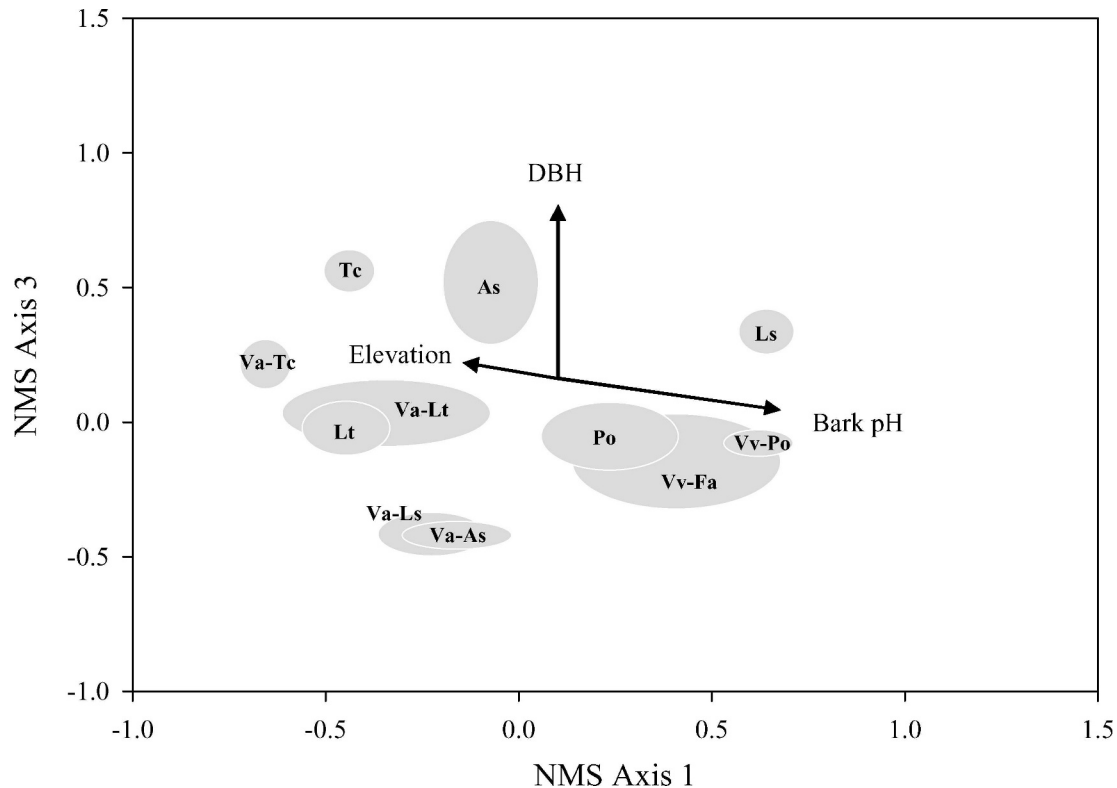
FIG. 1. Nonmetric multidimensional scaling (NMS) joint biplot of tree and grapevine species associated with environmental parameters. Abbreviations: DBH = diameter at breast height, and species abbreviations are the first letter of the genus and specific epithet, with neighboring grapevine species abbreviations followed by neighboring tree species abbreviation.

significant differences in similarity of myxomycete species assemblages. *Vitis aestivalis* neighboring *L. tulipifera* had the fewest (3) significant differences when compared to all other host species, followed by *L. tulipifera* (4).

Examination of the similarity between *V. aestivalis* neighboring different tree species showed that the greatest similarity (26%) was between those neighboring *A. saccharum* and *L. styraciflua.* Comparison between *V. aestivalis* neighboring the same tree species averaged 26.8%, yielding higher similarity between grapevines when neighboring the same tree species than when neighboring different tree species. The least similar *V. aestivalis* were those neighboring *A. saccharum* and *F. americana,* while all others were 14–24%. Comparing grapevine species *V. vulpina* neighboring *F. americana* and *P. occidentalis* showed 36% similarity, greater than between any of the combinations of *V. aestivalis.*

Among the species with a sample size of five, the species that had a greater difference in bark pH resulted in the greatest number of significant differences in community similarity. For example *V. aestivalis* neighboring *T. canadensis* resulted in significant differences (12) compared with all other

host species and *V. vulpina* neighboring *P. occidentalis* had significant differences with all (10) except *V. vulpina* neighboring *F. americana* and *V. aestivalis* neighboring *F. americana.*

Ordinations.—Nonmetric multidimensional scaling showed grouping of sites according to myxomycete species presence and absence and each was significant, supporting the hypothesis that distinct species assemblages are associated with tree or grapevine species. Joint biplots show the grouping of sites and the associated environmental parameters represented by vectors that are determined by the Pearson and Kendall Correlation, where the strength of the association is relative to vector length.

NMS was run with 138 myxomycete species and 27 tree and grapevine species, 40 runs with real data, 40 runs with randomized data, and resulted in three axes with a final stress value of 12.34, final instability of 0.017, with 50 iterations. In this analysis myxomycete species presence-absence data were associated with environmental parameters (bark pH, elevation, DBH, location in Kentucky or Tennessee, canopy height, tree species and grapevine species grouped by neighboring tree species) (FIG. 1). The greatest
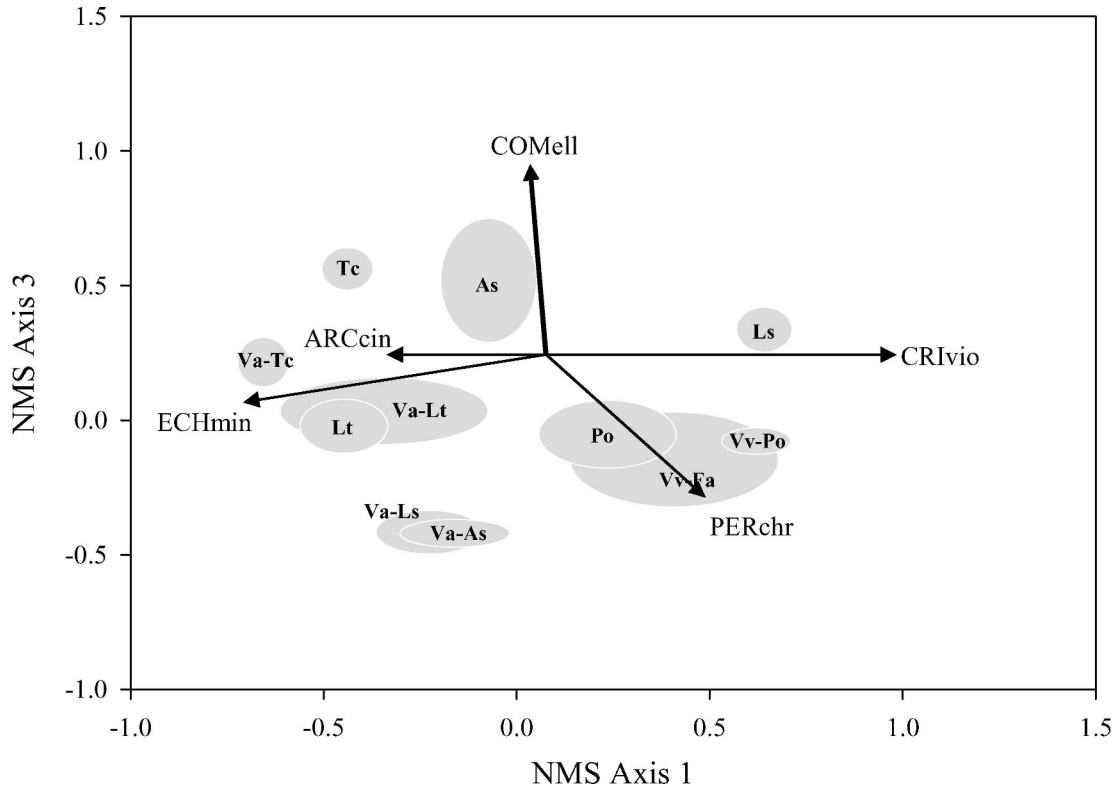
FIG. 2. Nonmetric multidimensional scaling (NMS) joint biplot of tree species and myxomycete species. Tree species abbreviations are the first letter of the genus and second letter of the specific epithet. Myxomycete species abbreviations are the first three letters of the genus in caps and the first three letters of the specific epithet in lowercase.

amount of variation among the four dimensions was observed along Axis 1 and Axis 3, (FIG. 1). Pearson and Kendall Correlations of environmental factors with ordination axes 1 and 3 (FIG. 1) as vectors with r^2 cut-off value of 0.150. Sites distributed along Axis 1 were associated with bark pH (tau = 0.444) and elevation (tau = −0.283), while along Axis 3 sites were associated with DBH (tau = 0.315).

Sites categorized by tree and grapevine species were graphically represented as bound by the standard error of the mean group distribution for the host species. Group distributions showed that each tree species had a distinct species assemblage of myxomycete species, indicating distinct species assemblages related to tree species. The only tree and neighboring grapevine that showed no difference was *V. aestivalis* and *L. tulipifera*. No difference was measured with respect to *V. vulpina* neighboring tree species, however, with the exception of *V. aestivalis* neighboring *L. styraciflua* and neighboring *A. saccharum*, differences were found with respect to every *V. aestivalis* neighboring a different tree species. Myxomycete species associated with each axis also were plotted but results did not group myxomycete species based on a particular taxonomic group or character; therefore results were not shown.

Myxomycete species were associated with sample sites and tree species. Association of myxomycete species with sample sites grouped by tree species is graphically represented with an NMS joint biplot (FIG. 2). The myxomycete species that shows the strongest correlation with Axis 1 is *Cribraria violacea* Rex (tau = 0.62) and with Axis 3 is *Comatricha ellae* Härk. (tau = 0.49). In addition three other myxomycete species are shown, two of which are more associated with Axis 1, *Echninostelium minutum* (tau = −0.67) and *Arcyria cinerea* (Bull.) Pers. (tau = −0.42) and *Perichaena chrysosperma,* which is associated with both axes 1 and 2 with tau = 0.40 and −0.41, respectively.

Multiresponse permutation procedure (MRPP) analysis was performed for tree and grapevine species, elevation, pH and sample height and are in agreement with the NMS results. Results of MRPP analysis showed host species, elevation and pH were significantly different among groups ($P < 0.05$), and had within-group agreement (A) of 0.28, 0.37 and 0.08 respectively and Test statistic (T) of −33.8, −16.9 and −7.4, respectively (TABLE V). No significant difference was found with respect to height in the canopy.

Indicator species analysis (ISA) was used to determine statistically important species on tree and

TABLE V.   Multiresponse permutation procedure (MRPP) test for grouping by tree and neighboring grapevine species, pH, elevation and height in canopy

	tree/vine species*	elevation*	pH*	height
Number of samples	209	120	120	120
Number of species	46	42	42	42
Test statistic (T)	−33.82	−16.87	−7.40	1.54
Observed delta	0.36	0.31	0.46	0.51
Expected delta	0.50	0.50	0.50	0.50
Variance of delta	0.00	0.00	0.00	0.00
Skewness of delta	−0.40	−0.29	−0.51	−0.77
Group agreement (A)	0.28	0.37	0.08	−0.01

*Results of the MRPP that were significant among groups (P < 0.05).

grapevine species. Results of ISA are provided (TABLE VI) and are in descending order of indicator value (IV). Statistical significance of each value was determined for species with a Monte Carlo test and found that the 21 species out of 44 tested are statistically significant in their association in each grouping. Some species with the greatest importance are *Dianema* sp. (IV = 58.3), *Comatricha ellae* (IV = 40.8), *Cribraria confusa* Nann.-Bremek. & Y. Yamam. (IV = 34.4), *Physarum crateriforme* Petch (IV = 34.0),

TABLE VI. Statistically significant myxomycete species associated with host trees and neighboring grapevines as determined by indicator species analysis

Myxomycete species	Max	Indicator Value (IV)	Mean ± SD*
Dianema sp.	2	58.3	5.3 ± 3.09
Comatricha ellae	6	40.8	7.2 ± 2.77
Cribraria confusa	6	34.4	5.5 ± 3.22
Physarum crateriforme	2	34.0	5.4 ± 3.13
Physarum nutans	2	32.4	5.9 ± 3.21
Lamproderma biasperosporum	11	29.0	5.7 ± 3.15
Clastoderma debaryanum	10	21.9	5.5 ± 3.10
Badhamia rugulosa	9	20.0	4.8 ± 3.39
Perichaena chrysosperma	12	18.0	7.6 ± 2.58
Cribraria violacea	2	17.3	7.2 ± 2.62
Licea parasitica	9	16.4	6.4 ± 2.86
Licea kleistobolus	4	16.2	4.8 ± 3.29
Echinostelium coelocephalum	8	16.1	6.0 ± 3.08
Cribraria minutissima	6	16.0	4.8 ± 3.50
Arcyria cinerea	10	15.7	8.2 ± 3.47
Echinostelium minutum	10	15.3	9.1 ± 1.94
Calomyxa metallica	11	14.8	5.5 ± 3.17
Enerthenema papillatum	2	14.7	5.2 ± 3.21
Perichaena depressa	9	13.9	5.0 ± 3.04
Clastoderma pachypus	10	12.8	5.6 ± 3.14
Diderma chondrioderma	12	12.4	5.0 ± 3.08

*SD = Standard Deviation.

Physarum nutans Pers. (IV = 32.4) and *Lamproderma biasperosporum* Kowalski (IV = 29.0).

Species assemblages of myxomycetes.—Assemblages on tree and grapevine species were determined with PC-ORD from a combination of NMS, MRPP, and ISA analysis by using the 21 species of myxomycetes that have associations with each host species (TABLE VII). *Liquidambar styraciflua* has the smallest species assemblage (six species) while *V. aestivalis* has the largest (16 species). The larger sample size of *V. aestivalis* (22 individuals, 106 sample sites) and *V. vulpina* (seven individuals, 34 sample sites) is responsible for some inflation of these lists but the relative number of myxomycete species per sample site (TABLE III) is relatively consistent with these findings, showing *Vitis* as a highly productive substratum in terms of myxomycete species.

DISCUSSION

Species assemblages of myxomycetes.—The first tree canopy study of myxomycetes by Snell and Keller (2003) examined five tree species up to 30 m from locations within GSMNP, whereas this study expanded this original research to include six tree species and corresponding grapevines with bark sampled up to 16.5 m from GSMNP, DBNF and BCF. Snell and Keller (2003) found that the distribution of species was patchy and some individual trees had a higher similarity with other species than with trees of the same species. In this study the Jaccard's similarity coefficient (TABLE IV) was used to examine myxomycete species assemblages on trees and neighboring grapevines and showed that the combination of *T. canadensis* and its neighboring grapevines, *V. aestivalis,* had the highest similarity (26%). This similarity was consistent with the hypothesis that grapevines and neighboring trees would have greater similarity between myxomycete species assemblages because

TABLE VII. Myxomycete species assemblages and abundance on each host species

	A. saccharum	F. ameri- cana	L. stryra- ciflua	L. tulipi- fera	P. occidentalis	T. canadensis	V. aestivalis	V. vulpina
Sample sites:	23	18	25	25	24	25	106	34
Arcyria cinerea	3	2	–	18	1	17	73	16
Calomyxa metallica	1	4	5	1	–	5	20	14
Clastoderma debaryanum	–	–	–	–	–	1	25	1
Clastoderma pachypus	–	3	–	1	–	1	27	1
Comatricha ellae	2	2	8	8	6	32	12	6
Cribraria confusa	3	14	28	1	10	2	34	46
Cribraria violacea	2	–	–	–	–	20	8	–
Echinostelium coelocephalum	–	–	–	–	2	–	37	5
Echinostelium minutum	3	2	–	26	7	23	118	11
Enerthenema papillatum	1	2	–	–	–	12	2	–
Lamproderma biasperosporum	2	–	1	1	1	3	12	14
Licea kleistobolus	–	–	–	8	–	–	4	–
Perichaena chrysosperma	2	3	7	2	10	–	59	43
Perichaena depressa	–	–	–	–	–	–	13	3
Physarum nutans	4	7	1	3	–	7	18	3

the hosts are nearby (within 3 m radius of tree) and are subject to the same environmental conditions. However the bark pH within species of *V. aestivalis* varied significantly. *Vitis aestivalis* neighboring *T. canadensis* showed a significantly lower bark pH than all other grapevines of the same species. The resiniferous substances exuded from *T. canadensis* likely were transferred during heavy rainfall and absorbed by the bark of the grapevine, thereby decreasing the bark pH, as is the case with soils under conifers. The lower bark pH of *V. aestivalis* neighboring *T. canadensis* resulted in a species assemblage of myxomycetes more similar to *T. canadensis* than the other *V. aestivalis* species, suggesting all other factors are less important than bark pH in affecting the occurrence of corticolous myxomycete species.

The species assemblages of myxomycetes of most tree species and neighboring grapevines had similarity of 15–20%, *A. saccharum* and its neighboring grapevines, *V. aestivalis,* had the lowest similarity (10%) and *T. canadensis* and its neighboring grapevines *V. aestivalis* showed the highest (26%). In cases where tree bark pH and grapevine bark pH are similar, the assemblages of myxomycete species were similar. Although there appears to be a trend between

a greater difference in bark pH and a lower similarity of myxomycete species assemblages, a linear relationship between the two is not clear (results not shown). These results suggest that additional variables need to be examined with respect to corticolous myxomycetes in the canopy and that bark pH might have varying levels of influence on the occurrence of myxomycete species within a community.

Distribution of myxomycete species.—Nonmetric multidimensional scaling (NMS), multiresponse permutation procedure (MRPP) and indicator species analysis (ISA) analyses showed that sample sites are grouped with significant differences in species assemblages according to host species (FIGS. 1 and 2). Testing the hypothesis of a random distribution of species across all sites with the ISA analysis yielded 21 species that were not randomly distributed and showed association with groups of sites. This complements the results of the NMS ordination (FIG. 2) that showed five species, *Arcyria cinerea, Comatricha ellae, Cribraria violacea,* and *Echinostelium minutum,* associated with the grouping of sites.

The NMS ordination (FIG. 1) showed species distributed along Axis 1 to have a greater association with a gradient in elevation and bark pH and those

TABLE VIII. Number of selected myxomycetes on trees and grapevines arranged by bark pH

	T. cana	V. aest (T. cana)*	L. tuli	V. aest (L. tuli)*	V. aest (L. styr)*	L. styr
n	5	5	5	5	5	5
pH ± SE	4.1 ± 0.08	4.5 ± 0.05	5.0 ± 0.03	5.2 ± 0.03	5.4 ± 0.1	5.8 ± 0.04
Cribraria confusa	20	5	0	2	0	0
Enerthenema papillatum	2	12	10	1	0	0
Licea marginata	0	0	1	3	0	0
Cribraria violacea	0	0	1	6	15	28
Macbrideola cornea	0	0	1	0	6	7
Perichaena chrysosperma	3	21	7	32	1	10

* Grapevine with neighboring tree species given in parentheses.

distributed along Axis 3 had a greater association with a gradient in DBH. For example *Clastoderma pachypus* Nann.-Bremek., *Dianema* sp., *Cribraria minutissima* Schwein., *Physarum nutans,* and *Enerthenema papillatum* (Pers.) Rostaf, are at one end of Axis 1 and *Cribraria violacea* Rex, *Stemonitis cf. curiosa* (ined published as *Stemonitopsis curiosa* Nann.-Bremek.) and *Physarum pusillum* (Berk. & M.A. Curtis) G. Lister are found at the other, indicating that the prior group is associated with low bark pH at high elevation, and the latter group is associated with hosts with high bark pH at low elevation. Species near the origin of the vectors, such as *Licea minima* Fr. and *Physarum synsporum* S.L. Stephenson & Nann.-Bremek., have little association with either gradient. However the association of sites with elevation covaries with bark pH because *T. canadensis* has acidic bark and occurs at high elevation, while *F. americana* has almost neutral bark pH and occurs in lowland, riparian zones. Diameter at breast height is also suspected to covary with host type, whether it is a tree or grapevine, and the diameter of mature trees also varies by species.

Indicator species analysis showed that almost half the corticolous myxomycete species observed in this study are not randomly distributed with respect to host species (TABLE VI). Comparing species assemblages of myxomycetes on each tree and vine species showed that *L. styraciflua* had the smallest species assemblage while *V. aestivalis* had the largest species assemblage. The larger species assemblage of myxomycetes associated with *V. aestivalis* is likely due to the greater variation in bark pH and larger sample size.

These results support studies that showed that certain myxomycete species occur on bark of a certain pH, here considered pH specialists, while other species occur on a wide range of bark pH and are here considered pH generalists (TABLE VIII). Furthermore species which occurred only once or twice are identified as more commonly occurring on the ground and are here considered opportunistic ground species. The adaptation of myxomycetes to bark pH might be attributable to the effect of bark pH on microbial food sources and the effect of bark pH on the cell membrane of the plasmodium or the process of sporulation (Gray and Alexopoulos 1968). Indeed Olive (1975) discusses the difficulty in culturing certain mycetozoans from the bark of living trees because they require a specific bacterial food source, such as *Escherichia coli.*

Vertical distribution of species.—This study also supports results of the first canopy study of myxomycetes showing no relation in the occurrence of species with respect to height in the canopy. *Platanus occidentalis* was an exception because it has unique bark characteristics, bark that flakes off with increasing height, thereby decreasing the occurrence of myxomycete species because of substratum loss. Bark near the base of the tree was thicker, spongier and more water absorbent, while bark near the top was actually the underlying bark layer that was exposed after the top layers flaked off. Bark near the top of the tree was thin, dense and somewhat hydrophobic and nonabsorbent. Although we recorded a slight change in pH with height, this might be attributed to the hydrophobic nature of bark near the top of the tree and therefore not an accurate reflection of bark pH. Furthermore results in this study suggest that variation in bark pH changes the species assemblage of myxomycetes, did not limit their occurrence and was therefore not suspect in the decrease in myxomycete species with increasing height.

Myxomycete species richness and relative abundance was lower in this study as compared to the previous study and can be explained by comparing myxomycete species occurrence. Many of the common species were present in both studies (*E. minutum, A. cinerea,* and *P. chrysosperma*), but in this study fewer rare species are represented by one to two collections. The sample size needed to record all

TABLE IX. Tree size and myxomycete species frequency compared to Snell (2002) unpublished data

Tree species	DBH ± SE	Frequency of myxo-mycetes (± SD)	Tree species	DBH ± SE	Frequency of myxo-mycetes (± SD)
Acer saccharum	59 ± 9.0	8.4 ± 3.5	*Acer rubrum*	69 ± 10	17.4 ± 4.5
Fraxninus americana	58 ± 5.9	24.4 ± 3.5	*Fraxninus americana*	71 ± 9.3	12.6 ± 4.0
Liquidambar styraciflua	77 ± 7.1	13.4 ± 3.3	*Liriodendron tulipifera*	124 ± 20	17.2 ± 3.9
Liriodendron tulipifera	66 ± 5.9	21.8 ± 8.1	*Pinus strobus*	79 ± 4.0	13.4 ± 4.1
Platanus occidentalis	54 ± 4.8	8.8 ± 1.6	*Quercus alba*	66 ± 7	17.8 ± 5.3
Tsuga canadensis	94 ± 9.1	37.0 ± 4.7			

possible species also is apparently large because *V. aestivalis* was represented by 222 moist chamber cultures and had more than three times as many myxomycete species observed than trees that were represented by 50 moist chamber cultures. Therefore the lower species richness in this study also might be attributed to the smaller sample size per individual tree (five versus 10 sample sites per tree reported by Snell and Keller 2003).

The difference in species richness and relative abundance also might be attributable to DBH. A clear difference was measured with respect to relative species abundance compared to that of Snell (2002) (TABLE IX). The first study indicated that rare species were identified as typical ground species that opportunistically developed on larger, older trees where the bark was in a preliminary stage of decay (Snell 2002). Indeed the trees sampled in the first canopy study were considerably larger, 40–180 cm DBH (mean 82 ± 6.5), as compared to this study, 37.5–125 cm DBH (mean 68 ± 3.7) (K.L. Snell pers comm). Future studies should test the hypothesis that species abundance of myxomycetes is greater on older, larger trees, suggesting a successional trend in myxomycete species assemblages with increasing age of the host species.

Conclusions.—This project was intended to answer questions concerning the patchy distributional patterns of corticolous myxomycetes on individual trees and across geographic regions. Grapevines on trees represented a situation where the distribution of myxomycetes on two individual host species in the same location could be compared in a vertical transect into the canopy, both within and across geographic regions.

The results support the findings of the first canopy study, showing bark pH and host species as factors that influence the species assemblages and distribution of corticolous myxomycetes. However grapevines of the same species neighboring different tree species had different bark pH. These results indicate that pH is the strongest factor influencing the distribution and occurrence of myxomycete species and species assemblages associated with host species. In addition these results also support the observation that some myxomycete species are pH specialists (low, medium or high bark pH) and others are opportunistic ground species or generalists. Bark pH is accountable for the observed regional variation in species, while patchiness on individual trees can be attributed to the life history strategy of corticolous myxomycete species, using small, water-conservative plasmodial types that do not migrate over distance (Everhart and Keller 2008). We recommend that future field research protocols should emphasize sampling adequate amounts of substrata in a local region from different host species with a wide range of bark pH, thus enabling researchers to obtain a representative sample of the corticolous myxomycete species for an entire region.

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