Evaluation of tree canopy epiphytes and bark characteristics associated with the presence of corticolous myxomycetes

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Abstract: Certain species of myxomycetes (plasmodial slime molds) are regularly present with mosses, lichens, and algae. Corticolous myxomycetes were previously studied in the tree canopy, and observations suggested that species occurrence is patchy and species abundance may increase with the presence of bryophytes and lichens. The purpose of this study was to quantify the association of corticolous myxomycete species with percent cover of epiphytes and with bark characteristics, such as water absorption, bark thickness, and bark pH. Study sites were located in three temperate forests in the southeastern USA. The doubled rope climbing method was used to collect bark from trees and grapevines in a vertical transect up to 15 m above ground level. Moist chambers (374) were used to culture myxomycetes for 32 d. The percent cover of lichens, bryophytes, myxobacteria, and filamentous fungi were estimated in five 2 cm × 2 cm quadrats for 187 sample sites. Results showed no association between percent cover of epiphytes and myxomycetes. Bark pH was the major factor influencing the occurrence of corticolous myxomycete species, and the patchy distribution of myxomycetes was attributed to the small plasmodium characteristic of most corticolous species.

Key words: bark, bryophytes, corticolous myxomycetes, Great Smoky Mountains National Park, lichens, tree canopy.

Résumé : On retrouve régulièrement certaines espèces de myxomycètes (moisissures plasmodiales visqueuses) associées à des mousses, des lichens et des algues. On a déjà étudié les myxomycètes corticaux dans la canopée des arbres et les observations suggéraient que leur distribution est éparse et que l'abondance des espèces pourrait augmenter avec la présence de mousses et de lichens. Les auteurs se sont proposé de quantifier l'association des espèces de myxomycètes corticaux avec le pourcentage de couverture par des épiphytes et avec des caractéristiques de l'écorce comme l'absorption d'eau, l'épaisseur de l'écorce et le pH de l'écorce. Les sites d'études étaient localisés dans trois forêts tempérées du sud-est des États-Unis. Les auteurs ont utilisé la technique d'escalade à deux cordes pour récolter l'écorce des arbres et des lianes, dans un transect allant jusqu'à 15 m de haut. Ils ont utilisé des chambres humides (374) pour cultiver les myxomycètes pendant 32 jours. Ils ont estimé le pourcentage de couverture des lichens, des bryophytes, des myxobactéries et des champignons filamenteux dans cinq quadrats de 2 cm × 2 cm, sur 187 sites d'échantillonnage. Les résultats ne montrent aucune association entre les pourcentages de couverture des épiphytes et les myxomycètes. Le pH de l'écorce constitue le facteur majeur qui influence la présence des espèces de myxomycètes corticoles et on attribue la distribution éparse des myxomycètes à la caractéristique de petitesse de la plupart des espèces corticoles.

Mots-clés : écorce, bryophytes, myxomycètes corticoles, Parc national des Great Smoky Mountains, lichens, canopée des arbres.

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Introduction

Myxomycetes (plasmodial slime molds) are protozoans characterized by haploid myxamoebae or biflagellate swarm cells that undergo syngamy followed by multiple nuclear divisions, developing into a multinucleate, assimilative, diploid protoplasmic mass called the plasmodium. At maturity, the plasmodium gives rise to fruiting bodies whose morphological characters are used for species identification (Everhart and Keller 2008). Myxomycete species are abundant and diverse within temperate forests, associated with rotting logs, leaf litter, dung, or the bark of living trees (Spiegel et al. 2004). Corticolous myxomycetes complete their life cycle, from spore to fruiting body formation, on the bark of living trees and woody vines (Keller and Brooks 1973).

Corticolous myxomycete species are known to occur in association with mosses, liverworts, lichens, and algae (Gray and Alexopolous 1968; Ing 1994; Keller and Braun 1999; Novozhilov et al. 2000; Schnittler et al. 2000; Smith and Stephenson 2007). Certain species of myxomycetes are found regularly with mosses and, for this reason, are termed bryophilous or bryophyte-inhabiting myxomycetes (Ing 1994; Novozhilov et al. 2000). Examples of bryophilous myxomycetes include *Barbeyella minutissima* Meylan and

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Colloderma oculatum (Lipert) G. Lister (Schnittler et al. 2000). Other myxomycetes, such as *Licea inconspicua* Brooks & Keller, are found on the lichenized bark of living trees (Keller and Brooks 1977).

Percent cover of epiphytes, macrofungi, and myxomycetes were examined on decaying logs on the forest floor and showed that lichens had a significant, negative influence on the occurrence of protostelids and myxomycetes (Rubino and McCarthy 2003). Bryophytes also showed a negative influence on the occurrence of myxomycetes and protostelids, however, it was not significant. Unfortunately, these results are not directly applicable to the occurrence of myxomycetes alone, since the protostelid *Ceratiomyxa* was included as a myxomycete. Furthermore, the results from surveys of decaying logs on the forest floor rather than the bark of living trees or woody vines would likely yield myxomycetes that are typical ground-site species, not corticolous myxomycetes. No studies have examined the relationship between corticolous myxomycetes and other epiphytes known to develop on the bark of living trees, such as filamentous fungi (molds), fresh water cyanobacteria of the genus *Nostoc*, and myxobacteria: bacteria that aggregate into fruiting bodies of the phylum Proteobacteria.

In addition to influencing the occurrence of certain epiphyte-inhabiting myxomycetes, epiphytic cover may also cause bark to retain more water, thereby increasing the abundance and occurrence of corticolous myxomycetes (Brooks et al. 1977). Various studies have examined bark water-holding capacity alone (Stephenson 1988; Novozhilov et al. 2000; Snell 2002). Snell and Keller (2003) found no significant difference in myxomycete species abundance with respect to bark water-holding capacity; however, studies by Schnittler et al. (2006) and Stephenson (1989) found the water-holding capacity of bark to be positively related to the occurrence of myxomycetes. These conflicting results may be due to varying amount of epiphyte cover on the hosts studied or differences in sampling methodologies.

Bark pH is known to influence corticolous myxomycete species, with certain species being specific to a certain bark pH range (ie. acidophilic species) and others being found over a broad pH range (Snell and Keller 2003; Everhart et al. 2008). In addition to bark pH, these studies showed that the occurrence of species on an individual tree is uneven or patchy, with species commonly found at discontinuous sampling heights within a single vertical transect. This may be attributable to variation in epiphyte cover or physical bark characteristics that were not studied. Indeed, it is possible that bark texture influenced myxomycete distribution by creating different microhabitats with varying degrees of spore adhesion (Stephenson 1988). However, no quantitative study of bark texture related to occurrence of tree canopy myxomycetes has been done.

Most studies of corticolous myxomycetes have examined bark within reaching distance on the trunks of living trees. However, fruiting bodies of ground-dwelling species like *Fuligo septica* (L.) F.H. Wigg. and *Lycogala flavofuscum* (Ehrenb.) Rostaf., were found at least 2 m above ground level on the bark of living trees and woody vines (Keller and Braun 1999). To avoid the transition zone between ground and corticolous species, the tree canopy has been redefined as 3 m and above (Snell and Keller 2003). In addi-

tion, lichens (McCune et al. 2000; Fanning et al. 2007), water-holding capacity, and bark thickness (Levia and Wubbena 2006) are known to vary with respect to height in the canopy. Therefore, the objective of this study was to determine whether epiphytic cover, bark thickness, and water-holding capacity influenced the occurrence and distribution of corticolous myxomycete species in a vertical transect into the tree canopy.

Materials and methods

Study areas

Three temperate forests in southeastern USA were selected for this study because of the diversity of myxomycete species documented in the region (Cooley 1934; Linder 1941; Welden 1951; Ford 1978; Branson 1988, 1990; Skrabal et al. 2001; Stephenson et al. 2001; Snell et al. 2003), favorable climatic conditions, variety of habitats, and a high diversity of tree and woody vine species, especially grapevines (*Vitis* spp.; Stupka 1964; Jones 2005). The Great Smoky Mountains National Park is located in the southernmost range of the Appalachian Mountains, on the state border of North Carolina and Tennessee, covering an area of approximately 210 545 ha (est. 1930, Shanks 1954). The Daniel Boone National Forest is in eastern Kentucky and north of the Great Smoky Mountains National Park. It spans 225 km from north to south on the Cumberland Plateau, west of the Appalachian Mountains, and encompasses an extensive, rugged area of over 258 440 ha (Collins 1975). The Berea College Forest is privately owned and managed by Berea College, Berea, Kentucky. The forest is located to the east of the Daniel Boone National Forest, near Berea, Kentucky, also on the edge of the Cumberland Plateau, and encompasses 3200 ha (Perry 2000).

Field methods

Field expeditions were conducted during the summer of 2006 in the Great Smoky Mountains National Park from 1–13 and 25–29 June, and 1–9 August, and in the Daniel Boone National Forest and the Berea College Forest from 15–23 June and 18–30 July. Field expeditions involved collecting myxomycetes from ground sites, but focused on sampling bark from the tree canopy for moist chamber culture. The doubled rope climbing method was used to access the canopy as previously described in detail (Jepson 2000; Keller 2004; Kilgore et al. 2008).

Suitable climbing trees (minimum DBH 60 cm), supporting a neighboring grapevine (minimum DBH 4 cm), were climbed and sampled. Only trees that allowed bark collection from the tree and grapevine in a vertical transect, every 3 m, from 3–15 m were sampled. The collection of bark involved evenly prying off bark samples from areas within reaching distance of the climber, avoiding damage to living tissue, and half-filling a paper bag (ca. 1000 cm^3). Every collection bag was labeled with the identifying number, tree or vine designation, and vertical height of bark sample.

The height of each tree was measured using 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 at a height approximately 6–

10 m on the opposite side from the trail or road. Ground-crew members were responsible for recording a datasheet of information with the identification number, tree species, universal transverse mercator coordinates, elevation, tree DBH, grapevine DBH, height of the tree, and general observations.

Leaf voucher specimens were collected for both the tree and grapevine. Grapevines were often difficult to reach owing to their growth habit, often only producing leaves above the outer canopy of treetops. When the grapevine 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.

Laboratory methods

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, met the minimum sample size of five individuals per species, and were represented at each study site.

Bark samples from five trees of each species and their corresponding grapevines, *Vitis aestivalis* Michx. or *Vitis vulpina* L., were used to prepare individual moist chamber cultures. Moist chamber cultures were prepared for each tree and associated grapevine at the same time using sterile, plastic Petri dishes (150 mm × 25 mm). Bark samples were randomly selected from collection bags. Ten moist chamber cultures were prepared for each tree and each grapevine, with two replicates for each height, here referred to as a sample site (Snell and Keller 2003). Maximum thickness and length of sampled bark was measured on five randomly selected pieces for each sample site.

Bark was placed in a single layer on pH neutral, sterile, P8-grade circular filter paper (150 mm diameter) inside each Petri dish. Bark was wetted directly with 35 mL of sterile deionized water that was adjusted to a pH of 7.0. Moist chambers were incubated under ambient light and room temperature (23–25 °C). After 24 h, pH was measured with a flat probe pH meter in three random places on the filter paper, close to the bark or under bark pieces. An additional pH measurement was taken for water prior to decantation, with the Petri dish tilted at a 45° angle. Water decant was measured by pipetting off free water that accumulated when the dish was tilted.

On days 4, 8, 16, and 32, samples were scanned for the presence of myxomycetes using a dissecting stereomicroscope at 70× magnification. The presence or absence of each myxomycete species for each moist chamber culture was recorded. A pin was placed near immature myxomycetes and mature myxomycetes were identified using a key to species by Martin and Alexopoulos (1969). Rare or possible new species were removed from moist chamber culture before day 32, prior to mold growth.

Percent cover data were collected in a stratified random sampling design for five individual trees of each species and for five selected individuals of each grapevine species, for a total of 374 moist chamber cultures. Sample quadrats were delimited on the lid of a Petri dish, dividing it into quadrats (4 cm²) using a straight-edge ruler and permanent marker. Partial quadrats and those touching the outer edge of the Petri dish lid were removed from the sampling area. There were a total of 21 quadrats per moist chamber culture, and two replicate moist chamber cultures per site, for 42 quadrats per each height on each tree and grapevine. Within each quadrat, the field of vision for the microscope at 70× magnification was used as the sample area (2 cm diameter). On day 32, five quadrats from each height were chosen at random and percent cover was visually estimated and recorded. Percent cover was measured for filter paper, mosses, liverworts, lichens, cyanobacteria, molds, myxobacteria, plasmodia, and myxomycete fruiting bodies. Also measured was percent cover of lichens when subdivided into crustose, fruticose, and foliose growth forms that are described by Brodo et al. (2001).

Voucher specimens of myxomycetes were made after all measurements were complete, 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 collection box, with standard information (Keller and Braun 1999) and sent to the United States National Fungus Collections (BPI), Beltsville, Maryland.

Data analysis

Raw data on myxomycetes included species richness and species abundance, where one or many sporangia of a species within a moist chamber culture dish was considered a single observation. The mean and standard error were calculated for both species richness and abundance for each tree and grapevine species. Analysis of vertical variation was first restricted to trees and grapevines of the same species. Myxomycete species richness at each height was compared with 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.

Data were tested for normality with Anderson–Darling test ($\alpha = 0.05$) and homogeneity of variance using Levine's test ($\alpha = 0.05$). Data that failed to meet the assumptions of Analysis of Variance, lacking normal distribution of samples and homogeneity of variance, were compared for significant differences using Kruskal–Wallis nonparametric ANOVA with Bonniferoni correction for multiple comparisons (Zar 1999). Where data were nonindependent and failed to meet the assumptions of a nonparametric ANOVA, significant difference was determined as nonoverlapping standard error of the mean. All descriptive statistics were calculated using SigmaPlot version 9.0 (Systat Software Inc., San Jose, Calif.), and comparisons were made using Minitab Release version 14.13 (Minitab Inc., State College, Penn.) and SPSS 12.0.1 statistical software (SPSS Inc., Chicago, Ill.).

Measures of bark thickness were first averaged for each height for each tree species and compared. Finding no difference, bark thickness was pooled and averaged for each species. Water decant was used to estimate water absorbed by the bark, here used as an indirect measure of water-holding capacity. Mean water absorption of bark was compared first at each sample height of each host species and

with no difference found, pooled, and compared by host species. Mean bark pH and SE were determined by converting measurements to hydrogen ion concentration (Guare 1991; Everhart et al. 2008). Owing to the non-normality and lack of homogeneity of values on the pH scale, the previously calculated means and SE values were used to determine significance, where nonoverlapping SE ranges indicated significant difference.

Analysis of percent cover data was performed by first removing percent cover of filter paper, thereby routinely scaling remaining data to reflect percent cover of bark. Because percent cover estimates lead to observer bias, the data were converted to cover classes to reduce this bias. Percent cover data were divided into six cover classes that were approximately equal in sample size ($n \approx 300$): 0% to 1.15%; >1.15% to 3.00%; >3.00% to 4.50%; >4.50% to 9%; >9% to 20.50%; and >20.50% to 100%. The average was determined using midpoint conversion for each cover class (0.575, 2.075, 3.75, 6.25, 14.75, and 60.25). Vertical variation in percent cover data was tested using regression analysis for each category and tested for significance with an ANOVA and Bonniferoni correction.

Indicator species analysis determined the constance or faithfulness of species assemblages to a group by weighted averaging. Each species was given an importance value (IV), obtained by combining the relative abundances and relative frequencies of epiphytes (Dufrene and Legendre 1997). Indicator species for each group or cluster are those with the highest absolute value of IV's. Significance was tested with a nonparametric procedure involving the Monte-Carlo permutation procedure (McCune and Grace 2002). Species with significant, nonrandom association with trees and grapevines were listed with their corresponding IV.

Nonmetric multidimensional scaling (NMS) was performed using PC-ORD statistical software (MJM Software, Gleneden Beach, Ore.) to determine relationships between myxomycete species and measured parameters. This nonparametric analysis was chosen because the data were nonnormal and lacked homogeneity of variance. The 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 analysis 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 between 10 and 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 showed the grouping of sites and either environmental parameters or myxomycete species as vectors related to the groups, where strength of the association to groups corresponded to length of the vector line and was 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).

Results

Bark characteristics

Bark pH, measured 1841 times, produced non-normal distributions, and lacked homogeneity of variance and independence when categorized by tree and grapevine species. Therefore, significant differences were determined by nonoverlapping SEM (Table 1.). There was no significant difference in pH measured near bark versus that when measured in the Petri dish tilted at a 45° angle for each tree and grapevine species. These measurements were combined at each sample height for each tree and grapevine. 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.

Significant differences were found with respect to bark pH based on species of tree or grapevine species, and when grapevines were segregated according to the neighboring tree species. *Tsuga canadensis* had the most acidic bark with a pH (4.1 ± 0.06) that was significantly lower than all the other species. The grapevine, *V. aestivalis,* neighboring *T. canadensis*, had the second lowest bark pH (4.5 ± 0.05) and was significantly different from all other host species. In contrast, bark samples of *F. americana* had the highest pH (6.3 ± 0.07) that was significantly different from all other host species. With the exception of *V. aestivalis* neighboring *T. canadensis,* all *V. aestivalis* bark samples had an average pH range from 4.8 to 5.4, with variation not attributable to the pH of the neighboring tree. The bark pH of grapevines of *V. vulpina* was higher than the pH of *V. aestivalis* and with a pH range from 5.5 to 5.7.

In addition to having the lowest pH, *T. canadensis* also had the thickest bark (9.3 ± 0.49 mm, Table 1.). Other tree species that also had thick bark were *F. americana* (8.5 ± 0.49 mm), *A. saccharum* (6.2 ± 0.61 mm), *L. styraciflua* (5.4 ± 0.28 mm), and *L. tulipifera* (5.0 ± 0.03 mm). *Platanus occidentalis* (2.7 ± 0.41 mm) had the thinnest bark and also showed significant difference with respect to height in the canopy (Table 2.). All grapevines had variable bark thickness and showed significant differences among individuals of the same species.

Increasing bark thickness is generally related to the amount of water absorbed by the bark but there were exceptions, for example, *T. canadensis* had the thickest bark and the least amount of water absorption (22.4 ± 1.0 mL, Table 1). *Platanus occidentalis* had the thinnest bark and second lowest water absorption (23 ± 1.6 mL), while *F. americana* (39.5 ± 1.9 mL) had the greatest water absorption and relatively thick bark. There were also significant differences in water absorption between individual grapevines associated with different tree species.

Epiphytes

Percent cover of bark by mosses, molds, liverworts, cyanobacteria, crustose lichens, foliose lichens, fruticose lichens, myxobacteria, myxomycete plasmodia, and myxomycete fruiting bodies was estimated in five quadrats (4 cm²) for 187 sites, for a total of 935 quadrats, and 10 287 estimates. After excluding sites devoid of epiphytes, the following remained: 6 sites from *F. americana*; 10 sites from *T. canadensis*; 23 sites from *A. saccharum*; 20 sites from *L. styraciflua*; 24 sites from *P. occidentalis*; 24 sites from *L. tulipifera*; 51 sites from *V. aestivalis* neighboring *A. saccharum, F. americana, L. styraciflua, L. tulipifera,*

Table 1. Mean bark pH, thickness, and water absorbed for host species in order of increasing acidity.

Tree or grapevine species	Bark pH	Thickness (mm)	Water absorbed (mL)
Tsuga canadensis	4.1a	9.3d	22.4f
Vitis aestivalis (*T. canadensis*)*	4.5b	3.5igh	23.8f
V. aestivalis (*Fraxinus americana*)*	4.8c	4.9c	—
Liriodendron tulipifera	5.0d	6.4a	30.4cd
V. aestivalis (*Acer saccharum*)*	5.1d	3.7fi	28.2ae
Platanus occidentalis	5.1de	2.7e	23.0f
V. aestivalis (*L. tulipifera*)*	5.2e	3.3h	26.7a
V. aestivalis (*Liquidambar styraciflua*)*	5.4f	3.9f	29.4de
A. saccharum	5.5f	6.2a	27.1a
Vitis vulpina (*F. americana*)*	5.5f	4.0f	31.5c
V. vulpina (*Platanus occidentalis*)*	5.7g	3.8fg	29.8d
Liquidambar styraciflua	5.8h	5.4c	31.7c
Fraxinus americana	6.3i	8.5b	39.5b

Note: Letters indicate significant differences in pairwise comparisons using SE.
*Grapevines have species name of neighboring tree given in parentheses

Table 2. Bark characteristics and myxomycete species richness for *Platanus occidentalis* bark samples taken at 3, 6, 9, 12, and 15 m above ground level.

Height (m)	Bark pH	Water absorption (mL)	Thickness (mm)	Species richness	n
15	5.2b	18.5c	1.2d	1c	4
12	5.2b	18.6c	1.8c	2b	5
9	5.0a	21.6c	2.2c	5b	5
6	5.12a	23.8b	3.3b	11a	5
3	4.9a	31.7a	4.8a	12a	5

Note: Letters indicate significant differences in pairwise comparisons using SE.

Table 3. Percent cover of bark by mosses, liverworts, cyanobacteria, crustose lichens, and myxomycetes (mean ± SE).

Species	Mosses	Liverworts	Cyanobacteria	Crustose lichens	Myxomycetes
Tree					
Acer saccharum	14.9±4.8	0	0	6.8±3.8	0.3±0.2
Fraxinus americana	36.1±10.9	0	2.6±2.4	0.6±0.6	3.0±2.4
Liquidambar styraciflua	19.9±6.2	0	0.2±0.2	5.1±3.4	0
Liriodendron tulipifera	13.9±3.8	0	0.1±0.0	16.6±4.8	0.8±0.3
Platanus occidentalis	3.3±3.2	0	0	4.1±3.2	0.4±0.2
Tsuga canadensis	2.2±1.5	0.6±0.1	0	19.4±8.9	0.3±0.2
Grapevine (tree neighbor)					
Vitis aestivalis (*F. americana*)	0.4±0.4	0	0	0	4.9±2.6
V. aestivalis (*L. styraciflua*)	2.1±2.1	0	0	0	8.9±8.6
V. aestivalis (*L. tulipifera*)	17.8±6.7	0	0	14.8±5.9	3.1±1.5
V. aestivalis (*T. canadensis*)	0.2±0.2	0.5±0.3	1.2±1.2	0.6±0.3	3.8±1.6
Vitis vulpina (*F. americana*)	0.9±0.7	0	0	9.6±3.2	5.1±2.7
V. vulpina (*P. occidentalis*)	1.4±0.8	0	0.1±0.1	15.6±4.7	2.9±1.0

Note: Letters indicate significant differences in pairwise comparisons using SE.

and *T. canadensis*; and 29 sites from *V. vulpina* neighboring *F. americana* and *P. occidentalis*. Sites lacking any epiphytes or myxomycetes were excluded from the data analyses.

Epiphytes, except fruticose lichens, were present at all heights for all host species (Table 3.). Fruticose lichens were not present at 3 and 6 m above ground. Regression analyses showed there was no significant difference within each category of epiphyte with respect to height in the canopy when analyzed separately by individual host species and when host species were pooled. However, significant differ-

ences were found when comparing species richness of myxomycetes at each height on *P. occidentalis*. Among all five *P. occidentalis*, there was a significant difference in species richness between three groups of sampling heights, the lowest heights (3 and 6 m; 12 and 11 species, respectively), the next two heights (9 and 12 m; 5 and 2 species, respectively), and the highest sampling height (15 m; 1 species).

There were significant differences found among all tree and grapevine species with respect to the percent cover of epiphytes and myxomycetes. Pooling data by tree and grapevine species showed mosses had the greatest cover (9.8% ±

1.5%), followed by cover of crustose lichens (9.2% ± 1.5%), foliose lichens (4.4% ± 1.1%), and molds (4.0% ± 0.9%). Fruticose lichens were only scantily present on one tree species, *L. tulipifera* (0.9% ± 0.9%), and were therefore significantly different than all other epiphytes or myxomycetes. Percent cover of myxomycetes (2.0% ± 0.5%) was more than fruticose lichens but not significantly different from percent cover of plasmodia (0.1% ± 0.05%), myxobacteria (0.08% ± 0.05%), cyanobacteria (0.2% ± 0.1%), or liverworts (0.07% ± 0.05%).

Comparisons of each category of percent cover converted to cover classes were made between host species and showed significant differences with respect to percent cover of mosses ($p = 0.000$), liverworts ($p = 0.004$), cyanobacteria ($p = 0.000$), crustose lichens ($p = 0.000$), and myxomycetes ($p = 0.002$). Moss coverage was greatest on *F. americana* (36% ± 11%). All others averaged 20% or lower. Mold growth was highest on *V. vulpina* neighboring *F. americana* (19.0% ± 11%), while all other host species averaged 10% or less. Liverworts were measured only on *T. canadensis*, and *V. aestivalis* neighboring *T. canadensis*, covering less than 1% of the area. Cyanobacteria were most abundant on *F. americana* (3.4% ± 3.3%), but were also present on *L. styraciflua*, *L. tulipifera*, *V. vulpina* neighboring *L. styraciflua*, and *V. aestivalis* neighboring *T. canadensis*, although all were less than 1.5%. Crustose lichens covered nearly 20% of *T. canadensis* (19.0% ± 10%). All other tree and vine species had less than 12.5% coverage, and crustose lichens were absent on *V. aestivalis* neighboring *A. saccharum*, *F. americana*, and *L. styraciflua*. Foliose lichens were most abundant on *V. vulpina* neighboring *F. americana* (18% ± 11%), but covered an average of no more than 13% on all other hosts. Fruticose lichens were only present on *L. tulipifera* at less than 1%. Likewise, myxobacteria were present only at less than 1% on *A. saccharum*, *V. vulpina* neighboring *P. occidentalis*, and *V. aestivalis* neighboring *L. styraciflua*. Plasmodia of myxomycetes were observed most on *V. vulpina* neighboring *F. americana* (1.4% ± 1.2%), and were observed less than 0.3% on all other hosts. Similarly, myxomycete fruiting bodies covered the greatest area on *V. aestivalis* neighboring *L. styraciflua* (8.9% ± 8.5%). Myxomycete fruiting bodies were the only category to be present on every host species surveyed.

Indicator species analysis and ordinations

An indicator species analysis (ISA) was run, on tree data only, to avoid mixed-effects of grapevines neighboring different tree species. There were 1000 permutations run to determine significance, where only mosses ($p = 0.0020$), cyanobacteria ($p = 0.0080$), crustose lichens ($p = 0.0090$), and myxomycetes ($p = 0.0070$) had significant associations with tree species. Among those four groups, *F. americana* had the highest indicator value associated with mosses (40), cyanobacteria (30), and myxomycetes (43), while *T. canadensis* had the highest indicator value for crustose lichens (33).

The ordination utilized nonmetric multidimensional scaling and showed grouping of myxomycete species (as sample units) according to tree or grapevine species, indicating distinct species assemblages associated with tree or grapevine species. Joint biplots showed vectors ($r > 0.15$) for bark pH,

bark thickness, elevation, host's DBH, and foliose lichens (results not shown). Analyzing the data for individual tree and grapevine species produced the same results as when data were combined. Lichen growth forms were also combined and rerun but did not produce a vector associated with myxomycete species presence. The relationships between vectors of bark pH, water-absorption of bark, and DBH of host species were analyzed with a multi-response permutation procedure and showed significance only with respect to bark pH (results not shown).

Discussion

The bark of 30 individual trees and 30 grapevines was sampled, and 580 moist chamber cultures yielded a total of 1324 myxomycetes observed, representing 46 myxomycete species and 19 genera, with an additional 2 taxa identified only to genus (Everhart 2007; Everhart et al. 2008). These data were analyzed further with information on bark characteristics and epiphytic cover to determine the influence of these factors on the occurrence of myxomycete species.

Results showed that the grapevine *V. aestivalis* neighboring the highly acidic conifer *T. canadensis*, had significantly lower bark pH compared with other grapevines of the same species neighboring different tree species (Everhart et al. 2008). This study showed that despite *V. aestivalis* neighboring *T. canadensis* having few significant differences in physical characteristics (thickness and water absorption, Table 1.) as compared with other *V. aestivalis*, the change in bark pH yielded a different species assemblage of myxomycetes, typical of acidic bark. This similarity in bark pH and species assemblage between neighboring tree and grapevine did not occur for any other pair of tree–grapevine combinations. In addition, the results of the NMS ordination or subsequent MMRP did not indicate bark thickness or water absorption as significant influences on the myxomycete species assemblages.

This study supports results of the first canopy study of myxomycetes showing no relation in the occurrence of species with respect to height in the canopy (Snell and Keller 2003). However, one exception to this observation was the result from *P. occidentalis*. There was a significant decrease in species richness of myxomycetes from bottom to top of *P. occidentalis*. Species richness of myxomycetes was not significantly different with respect to height in the canopy for the grapevines neighboring *P. occidentalis*, suggesting that the vertical variation observed for these trees was likely due to its unique bark characteristics. For *P. occidentalis*, bark thickness, water absorption, and pH showed significant decrease with height in the canopy (Table 2.). In addition, bark sampled near the base of the trees was thicker, spongier, and more water absorbent, while bark sampled near the top was actually the underlying bark layer that was exposed after the top layers flaked off. This bark near the top of the trees was thin, dense, and somewhat hydrophobic.

The significant change in bark pH with height in the canopy for *P. occidentalis* was considered as the cause for reduced species richness of myxomycetes. However, previous studies suggest change in bark pH was not likely to have reduced the occurrence of species, instead it would have changed which species were found there (Everhart et al.

2008). For example, grapevines growing alongside the conifer *T. canadensis* had a more acidic bark pH than grapevines neighboring other tree species. These grapevines had a collective species assemblage of myxomycetes typical of the conifers they neighbored. This change in bark pH of the grapevines was likely due to resiniferous substances of the conifer leaching onto and being absorbed by the bark of the grapevines. More importantly, the significantly lower bark pH of these grapevines did not reduce the number of species observed. Therefore, we conclude that in the case of *P. occidentalis,* the change in bark pH at higher sampling heights was not likely to have caused the observed decrease in myxomycete species richness.

The slight change in bark pH of *P. occidentalis* with increasing height in the canopy was more likely attributed to the hydrophobic nature of bark near the top of the tree, and therefore not an accurate reflection of bark pH. Furthermore, the hydrophobicity and smooth bark surface were probably the cause for fewer myxomycetes being observed higher in the canopy. Smooth surfaces of the upper canopy bark likely reduced spore adhesion. In addition, with little moisture absorbed by the bark, any myxamoebae or swarm cells present on the bark probably would not be able colonize much of the bark surface or survive for long periods of time. It is also questionable as to whether food sources (fungal spores, bacteria, and other microorganisms) for the myxamoebae and swarm cells would be present on this type of smooth, hydrophobic bark.

Examining the percent cover of epiphytes (mosses, liverworts, crustose lichens, foliose lichens, fruticose lichens, cyanobacteria, molds, and myxobacteria) yielded no associations with the presence of myxomycete species, percent cover of plasmodia, or percent cover of myxomycete fruiting bodies. This was partially explained by the highly variable dataset, with large variation in epiphytic cover based on the host species, some of which may also covary with bark characteristics. For example, *F. americana* had bark with the highest water absorption, highest pH, and also had the greatest coverage of mosses and cyanobacteria.

The ISA indicated moss, cyanobacteria, and myxomycete fruiting bodies as having the highest indicator value associated with *F. americana.* Similarly, the grapevines *V. vulpina* neighboring *F. americana*, had the third-highest bark pH, fourth-highest water absorption, and showed the highest value for percent cover of foliose lichens, molds, plasmodia, and myxomycete fruiting bodies. Although environmental factors may have influenced the association of these epiphytes with these trees and grapevines, these could not be effectively separated within this dataset since there were so few *V. vulpina* grapevines. It is more likely, however, that the similar bark characteristics of these two species (*F. americana* and *V. vulpina*) influenced this association. The bark of these species was spongy in texture and readily absorbed water, consistent with previous studies indicating that high bark pH and high water-holding capacity increases the presence of mosses (Studlar 1982).

In contrast, *T. canadensis* had the thickest and least water-absorbing bark, with the lowest pH, and was one of only two host species with liverworts (the other being *V. aestivalis* neighboring these trees). *Tsuga canadensis* also had the highest crustose lichen coverage and highest indicator value

for crustose lichens, consistent with these being larger, older trees (Everhart et al. 2008). Nevertheless, the NMS ordinations did not show an association between epiphytes and myxomycete communities, even when the analysis was restricted to a single host species.

It is suggested that future studies focus sampling effort on trees with different pH, such as *F. americana* and *T. canadensis*. These studies should select areas of the trees with high and low epiphytic cover for comparison. Individual myxomycete fruiting bodies should also be counted in lieu of percent cover due to the dramatic size variation among fruiting bodies of species such as *Perichaena chrysosperma* (Currey) A. Lister, which is much larger than *Echinostelium minutum* de Bary. Future studies should focus on the most abundant myxomycete species with medium to large fruiting bodies, such as *Arcyria cinerea* (Bull.) Pers., *P. chrysosperma,* and *Cribraria violacea* Rex, and survey the number of fruiting bodies per Petri dish for more than five individual trees of the same species at heights of 3, 9, and 15 m above ground level. Comparisons should also be made with myxomycete species categorized by plasmodial type, since phaneroplasmodial species would likely be much more common on highly water-absorbent bark with epiphytic cover, whereas protoplasmodial and aphanoplasmodial types would be more common on nonepiphyte inhabited bark. An effort to identify species of bryophytes, lichens, myxobacteria, and cyanobacteria should also be made to deepen our knowledge of microenvironmental community associations.

The results of this study were consistent with previous studies showing bark pH as the predominant factor influencing the community of myxomycete species (Snell and Keller 2003; Everhart et al. 2008). Examination of variation in percent cover of epiphytes, bark thickness, and the water absorption of bark failed to explain the uneven distribution of myxomycete species in the tree canopy. Results indicated that bark pH was the major factor determining the occurrence of most corticolous myxomycete species, relative to host species. The patchy distribution of myxomycetes was not explained by epiphytic cover or physical bark characteristics. Therefore, it is likely the patchy distribution patterns associated with corticolous myxomycetes are the result of the r-selected life history strategy, which favors species with small plasmodia that do not migrate over long distances and produce spores rapidly in tiny fruiting bodies (Everhart and Keller 2008). Although species-specific relationships with epiphytes may occur for certain myxomycete species, broad conclusions concerning the relationship between communities of corticolous myxomycetes and epiphytes could not be made.

Acknowledgements

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