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Distribution of the moss Orthotrichum anomalum on gravestones in Oakwood Cemetery in relation to rock type, microtopographic complexity and stone age

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Abstract:

Bryophyte species often live in very specific habitats based on a complex relationship between water and nutrient requirements and tolerance of environmental conditions. The objective of this study was to further define some of the conditions under which *Orthotrichum anomalum* will colonize a site. Since the study site was the anthropogenic habitat of gravestones within a city cemetery, the goal was to determine if colonization preference, substrate specificity in particular, for this moss would change within a manmade system. This study focused on the distribution of *Orthotrichum anomalum* as it relates to rock type (granite, limestone or marble), microtopographic complexity and stone age based on percent moss cover. Stones (n=99) from study sites (n=3) within Oakwood Cemetery were randomly selected to be sampled vertically via transect for percent cover of: the total stone, each aspect and each microtopographic class (vertical, horizontal, slant and curve). Stones were scored for microtopographic complexity and age was approximated from the death dates engraved on the stone. Statistical analyses were done to determine if there were significant differences in percent moss cover for: each rock type, the level of microtopography and the approximated age. These tests included: one-way ANOVA tests for rock type, microtopographic class and aspect: and linear regressions for microtopographic complexity and age. There was a significant difference ($p<0.0001$) in mean percent moss cover between granite and both limestone and marble, but not between marble and limestone. There was no significant relationship between microtopographic complexity and percent cover of *Orthotrichum* ($p>0.1$). Differences in microtopographic class preference were significant ($p<0001$). The Tukey test divided the classes into two groupings based on moss percent cover, horizontal coupled with slanted surfaces and vertical coupled with curved surfaces. Estimated age of the stone was not significantly

correlated with percent cover ($p>0.9$). My study supports preferential colonization of *O. anomalum* on calcareous rocks as reported by Crum (2004) further supporting the substrate specificity found in other studies (Gabriel and Bates 2005; Cleavitt 2001: Cleavitt 2002: Pharo and Beattie 2002). Though microtopographic complexity did not have a linear relationship with percent moss cover, that there were differences for microtopographic class brings up interesting questions about preferential positioning of moss species on substrates with and without competition. Percent moss cover variation over time could reflect clearing and/or stochastic dispersal events. That *O. anomalum* has similar colonization patterns on gravestones which are analogous to its natural rock habitats implies that substrate specificity is unaffected by human manipulation of the substrate.

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Acknowledgements:

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Introduction:

Mosses are often described by non-bryologists as fuzzy green stuff, but they are part of a diverse and adaptive group that are an important component of all terrestrial and most aquatic ecosystems-including anthropogenic habitats. Mosses have colonized a wide range of manmade settings from the sides of buildings and the cracks in sidewalks to industrial waste sites. Cemeteries in particular are often well colonized by mosses, even within urban areas. *Orthotrichum anomalum* is one such moss species that has readily adopted the anthropogenic habitat of gravestones within cemeteries. The presence of *O. anomalum* within this setting lends itself to a number of interesting questions about its distribution. The primary question is whether it would follow the distribution pattern it has in non-anthropogenic habitats, which is on limestone outcrops, within this unnatural setting; and if not how it differs from this natural pattern. Since *O. anomalum* is a dominant within the cemetery used for this study it represents a unique habitat in which to study the factors that influence this species' distribution.

The distribution of moss species is based on a complex relationship between environmental characteristics and the ability of these characteristics to facilitate water retention and nutrient uptake. The true mosses are generally small with leaves a single cell layer thick and as a result have an intimate relationship with their environment. The importance of this close association is seen in their ability to absorb and hold nutrients and their poikilohydric nature. It is this close association that causes their great substrate specificity.

Mosses have a high cation exchange capacity (CEC) leading to nutrient catchment and sequestration, as ions, including heavy metals, are absorbed and fixed in their cell walls (Lindo and Gonzalez 2010; Cleavitt 2001). Cations are important for growth and the combination of cations present for a moss to exploit can influence protonemal growth and gametophore development, both of which are important aspects of the moss lifecycle (Bates 2000). The protonemata is the initial stage of development from which the gametophyte grows and gametophores are the shoots that bear the male and female reproductive structures. These stages are of great importance to the moss lifecycle which is why accessing and keeping the right complement of cations is so important. In addition, the high cation exchange capacity of mosses allows them to take up these vital nutrients from very dilute solution. Coupled with this ability, mosses only require a fraction of the nutrient concentration of vascular plants (Glime 2006). The exception to this pattern of minimal nutrient requirements is species who favor a particular nutrient, such as calcium. These species are often distinguished by where they grow; i.e. those who need more calcium grow exclusively on calcareous substrates. According to Crum, *Orthotrichum anomalum* is one of these species as it is commonly found on calcareous rocks (2004). Calcium loving species are often referred to as calcicole bryophytes and they have an elevated CEC compared to other bryophytes likely due to elevated demand for this cation (Bates 2000).

Mosses are nonvascular so do not have internal structures for water and nutrient transport. Instead mosses absorb water and accompanying nutrients over their entire surface. This characteristic makes it necessary for them to be unprotected for optimal uptake of water from their surroundings so they lack, with few exceptions, the cuticle that vascular plants use to protect themselves from water loss. Mosses can photosynthesize and grow only when both moist and illuminated, so water is the most common factor limiting establishment and growth. Mosses have the benefit of being poikilohydric. This means that during times of water deficiency they can dry out and essentially become dormant: after water conditions improve they rehydrate and resume photosynthetic and metabolic functioning (Lindo and Gonzalez 2010). This ability varies based on the relative moisture conditions in which they live; those species that live in perpetually moist environments cannot overcome drying and will die. There is also nutrient leakage with rewetting which negatively affects the plant so having a perpetually wet environment is best (Lindo and Gonzalez 2010; Cleavitt 2001). To prevent water loss and/or increase retention mosses have developed a variety of mechanisms and habits (Glime 2006). Water retention tactics can be as simply elegant as the crowding of shoots to facilitate capillary action or as complex as the use of specialized cells for complete shifts in leaf position with changes in moisture conditions. *Orthotrichum anomalum* is a saxicolous (rock-dwelling) species which means it lives in a predominantly xeric habitat (Crum 2004). Therefore it has the ability to dry and rehydrate and has adapted characteristics common to xeric species such as the cushion life-form.

Moss species can be separated into two main groups based on growth form, pleurocarps and acrocarps. Pleurocarpous and acrocarpous growth forms can be differentiated along a moisture gradient with pleurocarps more susceptible to water loss. Growth form is an important trait for determining water holding capacity and would influence the chosen habitat for these groups (Mill and Macdonald 2005). Growth form is dictated by genetics but life form is attributed to the interaction of genetic traits, environmental conditions and phenotypic plasticity. *O. anomalum* is defined by Crum as an acrocarpous cushion forming moss (2004). Cushions are a life-form composed of tightly packed shoots which facilitate the movement of water through capillary action. This tight packing also increases the boundary layer resistance of the species (Syeinbjornsson and Oechel, 1992). The boundary layer is the area of still air in the immediate vicinity of a surface, and is an important protection zone for mosses. The dome shape of cushions also reduces the exposed surface area which is ideal in xeric habitats where any exposed areas would be susceptible to desiccation from wind and solar radiation (Syeinbjornsson and Oechel 1992).

Sexual reproduction in mosses is also closely related to water availability, without it male and female gametes cannot come together. The sperm must swim through a continuous film of water, even an infinitesimal break is an insurmountable barrier. Sexual and asexual reproduction allow for the movement of moss species into new habitats through dispersal of spores and propagules. Spores and propagules may have to endure high stress conditions when released from the microenvironment of the adult into the surroundings. Survivorship of these propagules influences maintenance and spread of the species (Cleavitt 2002). Many spores and vegetative propagules are dropped into the immediate vicinity of the parent plant and are unable to overcome the boundary layer and thus migrate to other suitable habitats, but a small fraction escape the layer of still air and disperse widely.

It is for the above reasons that most aspects of the moss life history and adaptations are designed for water retention or to overcome intervals of water shortage. This enhanced water storage can also have an influence on the surrounding microenvironment because the water is slowly released to the environment. For well-placed moss colonies this release of moisture can create a moist microenvironment in which they thrive, essentially by creating a pocket of favorable microclimate within their substrate's boundary layer which further retards water loss.

As a result of nutrient needs and water storage capacity mosses can be very specific in their habitat, though the degree of specificity varies. For particular species, in this case *O. anomalum*, some habitats can enhance and supplement their natural abilities while others can dampen these abilities or make them maladaptive. The range of habitats for mosses is quite diverse from very ephemeral habitats like dung to permanent habitats like rock. Many studies have been conducted to determine what factors define the distribution of mosses. In many of these studies, substrate has consistently proven to be important for colonization (Gabriel and

Bates 2005; Cleavitt 2001: Cleavitt 2002: Pharo and Beattie 2002). Most substrates can be differentiated by their own group of moss species, though level of specificity for these species may vary. For instance limestone has a unique and often greater complement of species compared to other rock types (Cleavitt et al 2009): there are observable differences between the bryophytes inhabiting calcareous and non-calcareous surfaces even in the same environment (Cleavitt 2001). Despite high specificity the presence of species with such affinities is not an absolute indicator of mineral presence as there are other environmental properties that could influence their presence at a site (Cleavitt et al. 2009). Some studies have shown that substrate specificity is more important in the establishment stage and not as necessary in adult plants (Cleavitt 2001). Even if adults do not express high specificity it is still important for mature plants because initial establishment and conditions during early development dictate adult distribution, health and reproductive success (Cleavitt 2002).

Substrate specificity is closely related to the specific nutritional needs of the species and/or their ability to overcome otherwise toxic concentrations of associated minerals (Cleavitt 2001). Specificity could also be related to nutritional inputs such as acid deposition which can be too harsh for some species to survive without neutralization by basic substrates (Cleavitt 2001). The inverse to this is that nutritional inputs could also be sufficient to overpower the influence of substrate, for instance droppings from bird colonies can be abundant enough to overwhelm the influence of the nutrients that could be derived from the nesting surface (Pentecost 1980). As mentioned above, gravestones serve as the substrate for this study since *O. anomalum* colonization favors rock and this is the most consistent source of this substratum type. In the study sites chosen for this study external input is not high enough to overwhelm the influence of gravestones; though birds frequent the area few droppings were found on the stones. Many do suffer from heavy weathering probably due to acid deposition (pers. comm.

Greg Flick, CPG) but since *O. anomalum* shows an affinity for calcareous habitats even in other environments, calcium rather than neutrality is more likely the reason it favors the limestone substrate for colonization.

Water availability can also to some degree be dictated by the substrate. Some substrates absorb more water and/or retain it longer; even seemingly impervious substrates like rock can have different water relations depending on mineral composition and porosity. Some minerals, which are the compositional elements of rocks, are more attracted to water and hold it longer (Pentecost 1980). Water relations are also strongly influenced by microtopography within a specific habitat type. Microtopographic features of the substrate are more important than general topography for mosses due to their relative size and because niche partitioning among species happens at the finer scale of meters and centimeters (Alpert 1986). Microtopography is generated by a combination of surface characteristics which include but are not limited to: aspect, slope, height and concavity. These characteristics can create areas whose subtle physical differences influence their ability to catch and hold water. Since mosses grow only when moist, we might expect moss distribution to be sensitive to microtopographic features which influence the capture and retention of water.

Microtopography retains water in part through the creation of microclimates that range in temperature, moisture and protection from exposure. Cooler temperatures produced by shading can decrease the rate of water evaporation. Microtopographic pockets can have increased humidity as water is released but does not escape the boundary layer created by this formation. Also escape from the desiccating sun and wind cannot be underestimated as an important mechanism for water retention. These subtle differences in mcrohabitat can be so vital to moss species that they can in part dictate their distribution. Despite the necessity of moisture, problems can arise where there is too much water as well, which may encourage the growth of algae which can outcompete mosses for resources.

Microtopography and weathering play an important role in initial establishment through spore and propagule catchment. Certain topographic areas such as crevices have a greater likelihood of catching and retaining wind dispersed propagules which can then establish in the moist microsite (Pentecost 1980). Roughness of the rock surface also facilitates catching spores, especially when compared to polished surfaces (Pharo and Beattie 2002). The gravestones in this study form a gradient of textures from rough weathered stones to smooth polished ones. Different microtopographic classes can also facilitate spread, aiding a species' ability to disperse vegetatively, and in the case of *O. anomalum,* form colonial turf mats.

The gravestones used for this study have both elaborate and simplified microtopographic features. Some of the gravestones have been designed to imitate natural rock formations while others have precise geometric contours. The geometric nature of most gravestones allows them to be easily described and categorized for their microtopographic complexity and to differentiate between microtopographic classes in order to evaluate which feature has a greater influence on moss presence and coverage.

Stability of a substrate is another important factor for habitat selection and is involved in life history patterns including colonial formation. On ephemeral substrates species dedicate their short time to reproduction, depending on the next generation for the maintenance of the species. On permanent substrates, like rock, precedence can be given to vegetative growth and expansion. *Orthotrichum anomalum* seems to be utilizing both these strategies through the formation of colonial mats and the prolific production of sporophytes. In the absence of competition with other species, either bryophytes or those of other taxonomic groups, a species such as *O. anomalum* could spread to occupy all suitable habitat that fulfills its niche requirements. However, though this rock environment would not change radically over a short time period, its longevity could potentially allow it to host a succession of species which could outcompete and replace this species (Pharo and Beattie 2002). Other species with lower spore production could take longer to colonize gravestones, but be better suited to the same niche as *O. anomalum* and therefore outcompete *O. anomalum* for this valuable habitat. The more hardy species could also invade occupied areas by replacing colonies of *O. anomalum* which have fallen or otherwise been removed. *O. anomalum* could also facilitate its own replacement by creating an environment ideal for another species, for instance through soil accumulation which would allow for soil dwelling species to colonize a previously inhospitable habitat. As studies have not focused on *O. anomalum,* its competitive ability and successional status are not known. Based on current observed conditions, it does not appear to have enough competition at present to be affected as it is a dominant in this system and few other species have been sighted on sampled gravestones. For the time being we can only speculate due to its propensity for a permanent substrate that over time this species would spread and its coverage would have a positive relationship with age.

Despite their high substrate specificity mosses can still colonize anthropogenic habitats, which indicates that these habitats are sufficiently similar to natural habitats that species do not differentiate between them. These anthropogenic sites can be considered ecological analogues and serve as alternative hosts for species to colonize (Lundholm and Richardson 2010). Mosses have quite readily adopted these new substrates as seen by the presence of *O. anomalum* within this cemetery field site. Lundholm and Richardson attest that hard surfaces commonly found in cities can serve a similar purpose as natural rock outcrops and cliffs (2010).

Orthotrichum anomalum is commonly found on limestone outcrops throughout the northeastern United States. If these outcrops are analogous to limestone gravestones, would *O. anomalum* readily accept them as a habitat? How would this species react to the presence of a second calcareous rock type that is less common within the local area? Would *O. anomalum* adopt granite gravestones which are outside its natural distribution because this rock type dominates at this site? These are some of the questions that this study sought to answer. Since substrate specificity and microtopography have proven to have influential roles in moss distribution within natural systems, this study sought to determine if this influence transferred to anthropogenic habitats.

Hypotheses:

My hypothesis for substrate specificity was that *O. anomalum* colonization would favor calcareous limestone and marble gravestones and exclude granite, thus maintaining its natural distribution patterns. It was further expected that overall percent moss cover would be highest on limestone gravestones followed by marble and that granite would have zero percent moss cover.

This study also sought to determine if there is a relationship between overall microtopographic complexity of gravestones and percent moss cover. My hypothesis was that there should be an absence of *O. anomalum* on sites with no or minimal microtopographic complexity since microtopography facilitates colonization and establishment, and that percent moss cover would increase with complexity. I further hypothesized however, that there may not be a positive linear relationship between percent moss cover and complexity because *O. anomalum* may specialize in a particular microtopographic class.

My final questions involved age; whether age was positively correlated with percent moss cover and if I could estimate the time it would take to establish a colony of a given size. For *O. anomalum* which lives on a non-transient rock environment that is climatically stable and free of competition, I hypothesized that spread, defined by increased percent cover, should have a positive linear relationship with age. The age for the site and many of the stones is known; therefore age and its effect on distribution can be considered and may in part be explained within a cemetery setting. This relationship cannot be completely explained as there are no definite dates for initial colonization only residence time of the potential substrate, but it may provide initial insights into the relationship between stone age and moss abundance.

Methods:

Field Site Descriptions:

Field data on distribution of *Orthotrichum anomalum* on gravestones was collected from Oakwood Cemetery in Syracuse, NY. Within the cemetery, 3 study sites (Lot 21, Lot 22, and Lot 52) were chosen based on the presence of the target species *O. anomalum* and to give a comprehensive view of this topographically diverse area. Lot 21 and 22 were hill sites which represented this topographic feature within the landscape. Two hill sites were used because these areas were the least uniform and I wanted to account for inherent differences within the site by increasing sample size. The flat site, designated as Lot 52 on the area map, was more similar throughout than the other two lots and representative of many of the flatter sites within the cemetery. Oakwood Cemetery is also not uniform in its vegetation causing differences in canopy cover and protection among the lots. Beyond general topography and vegetation, sites were chosen to be as similar as possible, including: the rock types present, the basic proportions of these rock types and the estimated age of the stones. The engraved death dates on gravestones were used to estimate the age of gravestones and sites, but these estimates were done with the understanding that there can be inaccuracies in using this method. Based on age estimates using death dates on gravestones I believe that major disturbances from burials have been minimal within the study sites in the last 50 years. The last major disturbance throughout the cemetery system was a storm in the 1990's which devastated the tree population. All sites still undergo general grounds maintenance as expected of such a space, and they are frequented by numerous people and their pets.

Hill Sites:

Lot 21 and 22 were adjacent and of similar aspect, slope and ecology. Both were west-facing and hosted a dispersed woodlot of primarily *Quercus alba* with the occasional *Acer, Taxus, Carya* and *Cornus* species. Canopy conditions ranged from open to closed. Ground vegetation consisted of manicured lawn composed of grasses and wide swaths of *Climacium dendroides*. Shrubs associated with gravesites were present but few. Remnant stumps from large trees, potentially killed in the 1990s, can also be found in these sites.

Flat Site:

Lot 52 was a flat plot situated in a level area on the eastern side of the hill from which the other sites were chosen. The site's vegetation consisted of perimeter trees, primarily *Ginkgo biloba*, and manicured lawn. All stones in lot 52 were exposed on at least one side to solar radiation and wind. The flat areas of the cemetery, including this site, are more intensely maintained via machinery such as lawn mowers and backhoes.

Gravestone Description:

Gravestones were of three compositional types: marble, limestone and granite. Since marble and limestone have similar mineral content, both gravestone types were composed of calcite (calcium carbonate: $CaCO_3$). Impurities that occur during the formation of limestone can cause differences in color and the development of veins in marble. This explains surface differences among marble markers, though impurities are present in both rock types. When limestone transforms into marble the calcite recrystallizes into larger interlocking crystals. As a result of this metamorphosis, marble is less porous, harder and denser than limestone (pers. comm. Greg Flick, CGP). The marble stones within study sites were probably originally polished, but at present they are similar in roughness to the limestone stones which are heavily weathered. Granite stones were not uniform in their mineral composition and consisted of a variety of granite types including grey granite and westerly granite which were commonly used in the early years of the cemetery.

Gravestones ranged in height from 25 cm to over 250 cm. Gravestones were excluded from the study if they were: level with the ground (height < 25cm), too small (vol. < 5000 cm^3), too large (house-like), covered/surrounded by vascular vegetation, or toppled. Exclusions were made because these constituted different habitat types and colonization regimes.

Orthotrichum anomalum was the dominant bryophyte on the stones but they also hosted *Anomodon attenuates, Hedwigia ciliate*, other unidentified mosses, and a suite of lichen species. Presence of other mosses was minimal, but lichen presence had a range from less than 5% to greater than 60% cover.

Distances between stones varied, with some areas of high stone density while others had a single stone in an area 10 m^2. Gravestones could be considered the only suitable habitat within an unsuitable matrix, since this species prefers calcareous rocks (Crum 2004).

Sampling:

Gravestones:

Gravestones included in the sample were selected by systematically establishing transects at 10 m intervals in Lot 21 and 22 and at 5 m intervals in Lot 52. All stones that were intersected by the transect line that met the criteria given above were included in the study.

Table 1: Summary of sampling totals for each lot.

Lot	Number of Transects	Number of Stones
21	8	20
22	10	37
52	10	42

Table 2: Summary of total number of gravestones sampled for each rock type.

Rock Type	Number of Stones Sampled
Granite	70
Marble	24
Limestone	5

Percent Cover:

Sampling line-intercept transect was done to determine percent cover of *O. anomalum* on each gravestone*:* sampling was systematic with intervals based on equal division of the width of each stone face into roughly 20 cm sections. Sample transects were established vertically on each face of all stones at intervals from 17 cm to 26 cm. Distance between transect lines was based on the dimensions of the stone, which included stone size and geometry, to ensure that each stone was represented as completely as possible. At least 2 and as many as 8 sample transects were established for each face of each stone. The exception to this was stones with sides less than 20 cm where one transect was the only viable option. The number of transects was proportional to the size of the stone and consistent between small and large stones, with the largest stones having the most transects. Any *O. anomalum* that intersected the transect line was counted, and distances along the transect line in centimeters totaled and then divided by the total transect length to get a proportion for percent cover.

O. anomalum was identified based on surface characteristics that included: cushion life form, dark green to greenish-black color, and chestnut colored sporophytes with rostrate operculum. This species was previously identified with Crum's key for mosses of the Great Lakes forests (2004). No other moss species present in the cemetery was similar enough to be confused for this species.

Rock Type:

Rock composition/ type was determined by obvious surface characteristics including: mineral content, crystal size and placement patterns, and color. If any part of the stone was of different rock type or composition that part was excluded from the study. Many of the marble stones had concrete bases which were not sampled.

Microtopography:

Each stone was scored for microtopographic complexity on a scale from 0-7. Scores were based on accumulated points from the following criteria: each unique microtopographic class (slant, horizontal, half curve, s-curve etc.) was one point, moderate weathering was one point, heavy weathering was two points, a small figure was two points, a large figure was three points, stones crafted to look like natural stone outcrops were seven points (unless they had a smooth or curved surface which gave them six and five points, respectively), and if the stone was polished one point was deducted from the total. This criteria was based on how each feature could contribute to moss colonization and establishment. Each microtopographic class would offer another option that may or may not facilitate *O. anomalum* colonization. Weathering created shallow topographic features, but they varied and were over the entire face of the stone, which is why even moderate weathering was given the score of one. Heavy weathering of stones created ridges, creases, cracks and freckled surfaces which is how it attained its score of two points. Figures were usually isolated to a certain area such as one side or the top of the stone, but in some cases were very elaborate offering many options for colonization. The more elaborate ones were given three points. The final criteria was given the highest point value as it simulated natural rock and had a wide range of microtopographic features. This range of features could not be easily defined but included variation in all the major characteristics found in a natural setting such as concavity, slope, and aspect. Polishing is a texture rather than a microtopographic feature but because it influences spore catchment necessary for colonization it was included in the score. Level zero constituted a stone which was flat-sided and without weathering. A stone with a level seven had any combination of the above characteristics with an accumulated score of seven or higher.

To further define the influence of microtopography, ten of the stones with *O. anomalum* present were randomly selected for more intense sampling based on microtopographic class. Microtopographic features were defined for each stone (i.e. vertical, horizontal, slant, half curve etc.). Then for each stone the original vertical transects (used for overall percent cover) were reestablished, but Instead of measuring the percent moss cover for the full transect, each transect was broken up into the previously defined microtopographic features. For instance, the percent moss cover for all the vertical surfaces was added together and divided by only the portion of the transect line which intersected vertical surfaces.

Aspect and slope orientation for each face were recorded. Aspect was determined via compass. Since sampling was divided by face this allowed for isolation of the percent moss cover for the four aspects of the stone. Totals for moss cover based on north, south, east and west orientation were obtained and averages calculated.

Age:

Age of the stone was determined by engraved death dates and if the stone contained more than one date a range was recorded.

Size and Lichen Cover of Gravestones:

Relative size of gravestones from extra-small (roughly 30cm in height and 10 cm x 40cm in width) to extra-large (roughly 250cm in height and 150cm in width) was attributed to each stone. Lichen presence was scored at 10% intervals by sight based on total percent cover of the stone.

Site Conditions for Gravestones:

Basic surrounding conditions were observed and recorded for each gravestone. This was done in an attempt to explain outliers for percent moss cover and colonization anomalies. Canopy cover was used for basic light conditions and was determined by tree position and branching patterns. Canopy cover was categorized as: no canopy, if no trees shaded the stone; light canopy, if branches were present but did not completely cover the stone; or full canopy, if the stone was entirely under the shade of a tree. The species of the trees that composed the canopy were recorded, along with the distance of each tree from the stone and the direction. Any trees that did not shade stones but were within 10 m of a stone were also recorded. Stumps that were within 10 m were recorded along with their relative size. Any other vegetation or structure that shaded or obstructed wind flow to a stone was recorded, including neighboring gravestones and large bushes. Relative plot position was recorded based on distance to the roads that bordered each lot and which transect line crossed it.

Statistics:

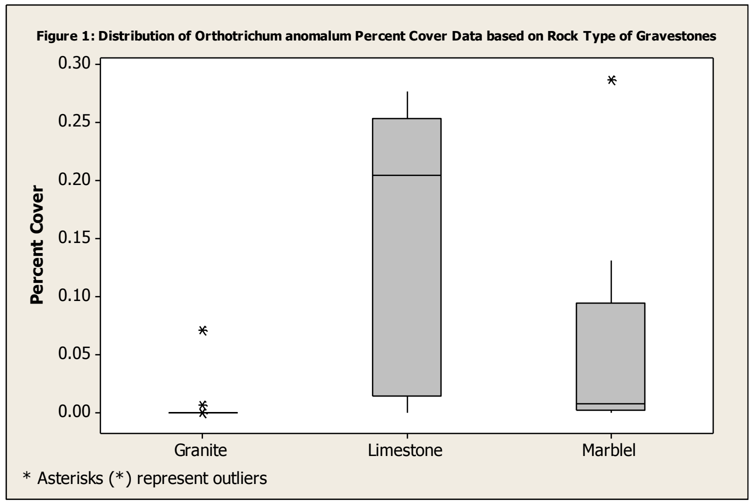
To compare percent moss cover of the three rock types a one-way ANOVA and associated Tukey test with a 95% confidence were used. A boxplot was generated to determine spread. To compare moss colonization among rock types, three 2-sample proportion tests were done using unique rock type combinations (marble-limestone, marble-granite, limestone-granite). Linear regressions were used to test the relationship between microtopographic complexity and percent moss cover, one for limestone and a second for marble. One-way ANOVA with Tukey was used to test microtopographic class and aspect. To determine if there is a relationship between age and percent cover a linear regression was done, stones with age ranges but no definitive date were not included.

Results:

Rock Type:

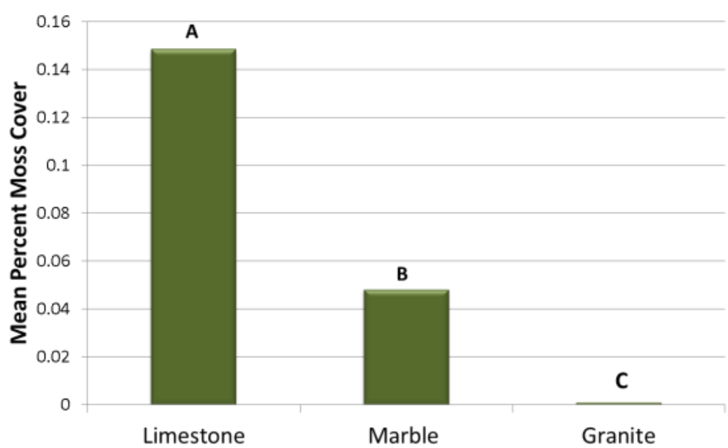
This study found a significant difference ($\alpha = 0.05$, p-value < 0.0001) among the three rock types for mean percent moss cover and a significant difference ($\alpha=0.05$, p-value < 0.0001)

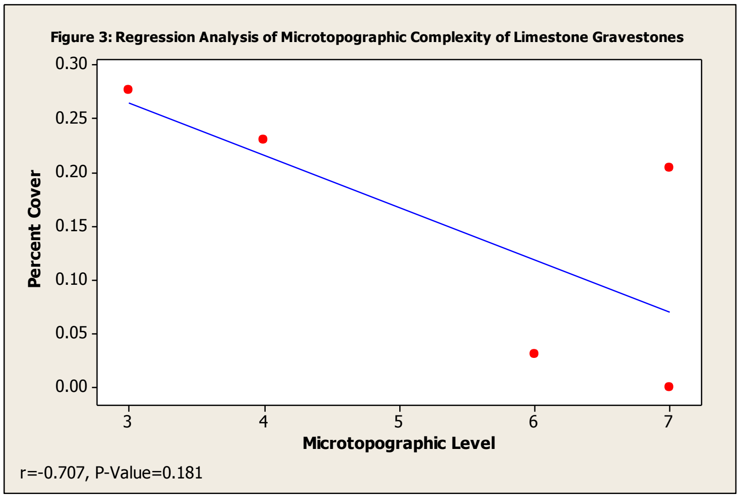
in moss colonization between granite and either marble or limestone stones. Figure 1 shows the averages and spread for the three rock type classes.



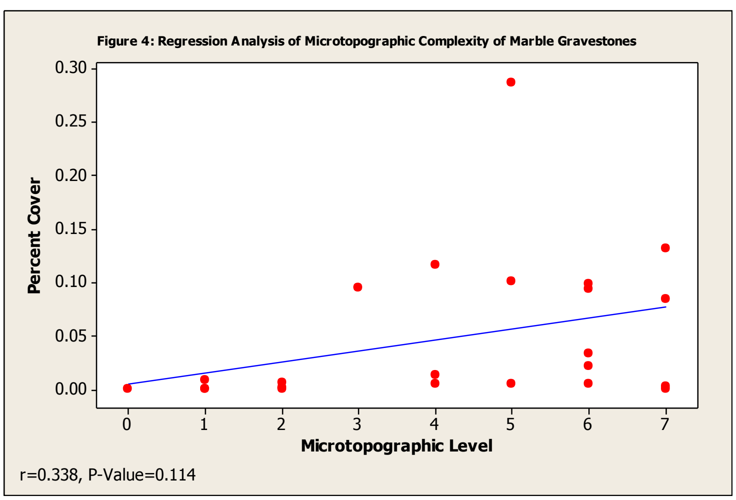
The influence of rock type on the establishment of *O. anomalum* was evaluated based on mean percent cover with ANOVA, which found a significance difference among all groups while the associated Tukey grouped the three types independently (Fig. 2). Limestone had the highest mean percent moss cover (14.9%) as expected based on its natural distribution, though cover varied from 0% to 28% for individual stones. Marble had the second highest mean percent moss cover (4.8%), and varied for individual stones similar to limestone from 0 to 29%. This study did not fully support my hypothesis about percent moss cover because some granite stones were colonized which gave this rock type a mean percent moss cover slightly greater than the zero cover predicted (0.12%).

Fig. 2: Mean Percent Moss Cover of *Orthotrichum anomalum* on Gravestones by Rock Type



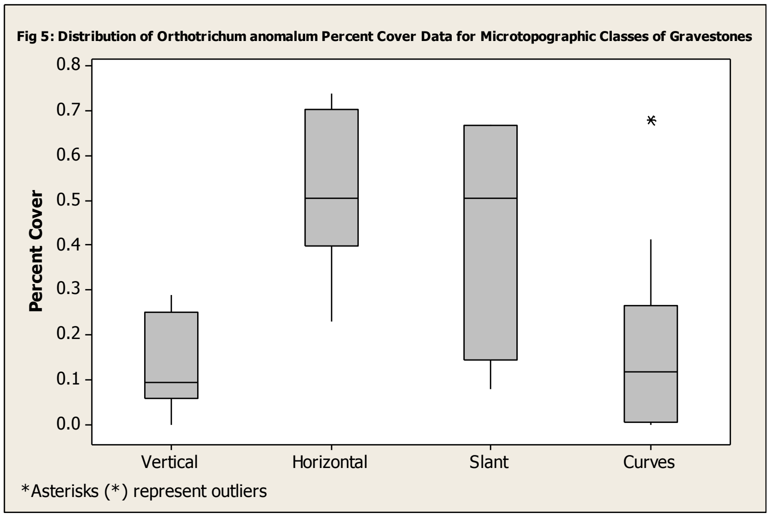


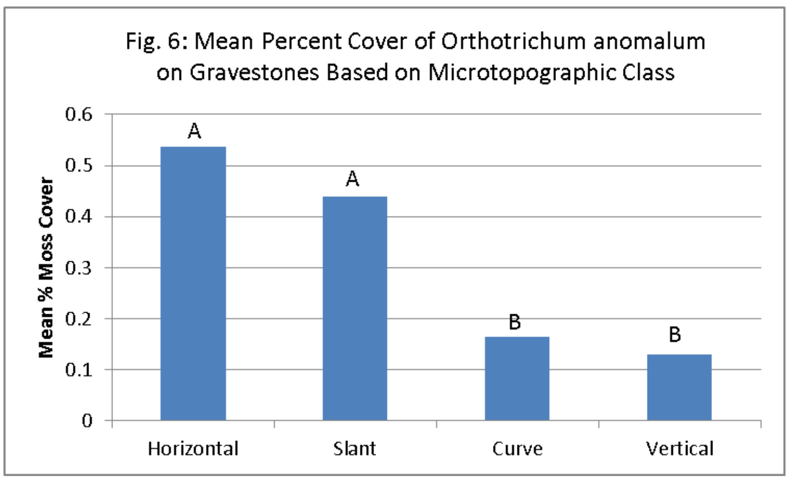
To determine if the proportion of moss colonized stones is equal among rock types I used a 2-sample proportion test which found a significant difference between granite and the two calcareous rock types, but not between the calcareous types (marble and limestone). This result was expected by *O. anomalum* based on Crum's description and supports my claim that the proportion of colonized granite stones would be lower than these rock types. This test did not support a significantly lower colonization rate in marble than limestone, indicating no colonization preference between these substrates despite differences in percent cover.

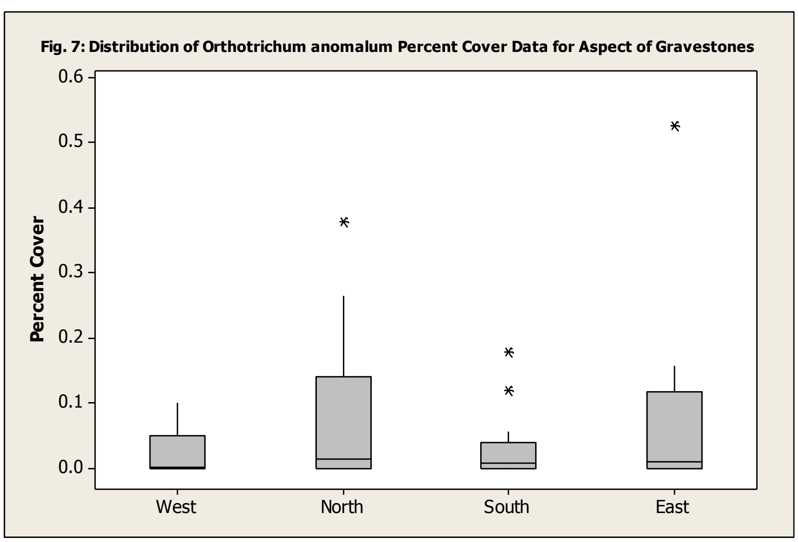


Microtopography:

This study did not support the claim that percent moss cover has a positive relationship with microtopographic complexity (α = 0.05, p-value >), but it did find significant differences (α = 0.05, p-value < 0.0001) in mean percent moss cover for microtopographic classes.

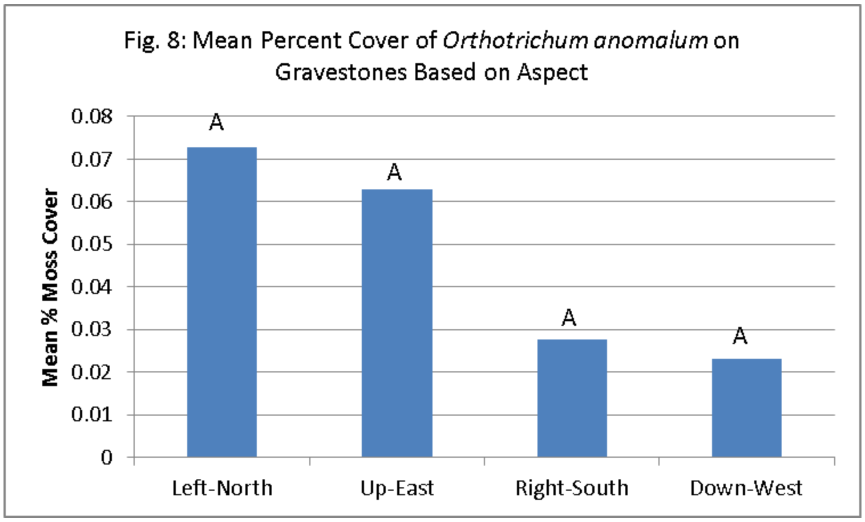






Linear regressions that were isolated by rock type (marble and limestone) were used to determine if there is a positive linear relationship between microtopographic complexity and percent cover. Neither regression evidenced a relationship between these variables (Fig. 3 and 4).

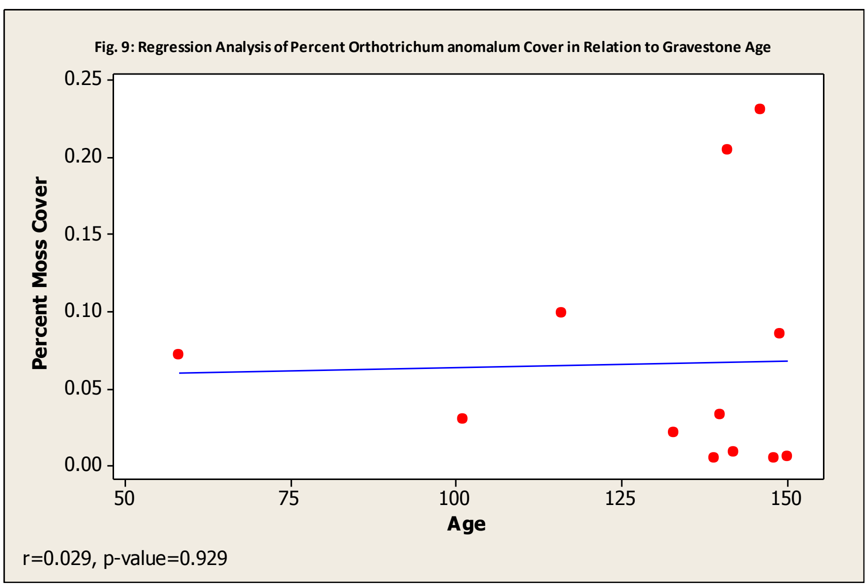
Further tests were done to determine if there were differences in mean percent cover for four main microtopographic classes: vertical, horizontal, slant and curve. The ANOVA indicated a significant difference (α = 0.05, p-value <0.0001) among these four classes. The Tukey test used 95% confidence intervals to divide these classes into two groups, with horizontal and slant in one group while curve and vertical were in the other (Fig. 6).



This result indicates that there is no significant difference between the horizontal and slant surfaces and between the vertical and curved surfaces in relation to colonization patterns.

The ANOVA for aspect did not support a significant difference (α = 0.05, p-value >0.1) in mean percent moss cover between faces. The means for each class followed the expected order observed in the field (fig. 7), but all faces fell within one group based on the Tukey test (Fig. 8).

Age:



This study did not find a relationship between the estimated age of the stones and percent moss cover (α = 0.05, p-value > 0.9). It was predicted that there would be a positive linear relationship between age and percent cover but this was not supported by the data (Fig. 9).

Discussion:

Rock Type:

Data on the distribution of *Orthotrichum anomalum* on gravestones did not support all hypotheses but there was significant evidence supporting a pattern of substrate specificity. The mean percent moss cover and colonization of *O. anomalum* were both significantly higher on marble and limestone than on granite, as expected from Crum's description of this species (2004). Substrate specificity is well recognized throughout scientific literature on moss distribution and community composition (Mills and Macdonald 2005; Pentecost 1980; Cleavitt 2001; Cleavitt 2002; Gabriel and Bates 2005; Pharo and Beattie 2002; Cleavitt et al. 2009). Despite statistical support for substrate specificity in this study there were some anomalies in the sites that require more in-depth study to determine the cause and could in part explain the lack of linear relationship between percent moss cover and both microtopographic complexity and age.

There was a great variation in percent cover *of O. anomalum* within the rock type groups. This variation within the rock types could have resulted from differences in compositional heterogeneity and/or differences in quality of the gravestones (Pharo and Beattie 2002). Granite gravestones were heterogeneous with differences in mineral content and placement, but this group was predominantly un-colonized. Heterogeneity in this group would not explain differences in the other rock type groups, but may explain exceptions within the granite group. The colonized stones could have had calcium as part of their mineral content: further tests would have to be done to know definitively. Marble and limestone gravestones were relatively homogeneous. Both were composed of calcite, but both groups had slight impurities. Otherwise limestone was uniformly composed of small crystals and marble slightly larger ones. Due to this nature in marble and limestone, differences in *O. anomalum* cover are likely due to stone quality. Quality encompasses a number of factors, in this case it could be related to weathering and/or the contributors to weathering altering the pH of the stones. Both calcareous rock types have suffered heavy weathering with acid deposition being a major contributor. This weathering process has the benefit of adding to microtopography and roughness, but weathering through acid deposition also constitutes an external input which could alter the suitability of the site. Acid deposition could cause conditions to become too acidic for *O. anomalum*.

With regards to the physical nature of weathering and the creation of roughened and ridged surfaces, the current level of roughness of the marble gravestones sampled could facilitate spread. However for aesthetic purposes the gravestones were originally smooth and the marble stones likely polished. This initial polished nature could have limited early establishment of spores and propagules as they more readily establish on rough surfaces (Pharo and Beattie 2002). Marble colonization and spread could have happened later as the original site conditions (slick, polished surfaces) were not conducive to the catchment of windblown propagules. This may account for differences in coverage between limestone and marble which had a lower average percent moss cover. Since marble and limestone are otherwise similar in mineral composition, the other explanations could be their crystal size and rock hardness or an as yet undetermined difference which requires further study.

This study did not find a significant difference in *O. anomalum* colonization between marble and limestone. Though this moss species is typically found on limestone outcrops and boulders, since there was no difference, marble could also serve as suitable habitat. Limestone preference may primarily be because of its availability within the range of *O. anomalum*.

Gabriel and Bates in a study about habitat specificity found low host/substrate specificity but that overall grouping of species was characteristic of substratum types (2005). For the purpose of this study marble and limestone are considered one substratum type classified as calcareous rock.

As with most data sets, there were unexpected colonization and lack of colonization events in this study. There was one limestone and six marble stones that were un-colonized out of a total of 29 calcareous stones sampled. Considering that these stones should be suitable habitat for *O. anomalum* according to substrate, some other characteristic of the stone or the environment in which the stone resides must be influencing its ability to be colonized. These events could have been influenced by their relative position or protection. Protection from the wind and any air blown spores and propagules that were circulating could have limited colonization. Over exposure to the desiccating sun could have also influenced colonization.

Despite selection for sites with similar gravestone rock types, type proportions and estimated age, there were inherent and influential differences between the flat and hill sites. The flat site was less protected relative to the hill site, which had the benefit of a west-facing slope and shade from vegetation. Differences in the hill and flat areas could account for the wide variation of percent cover for marble and limestone gravestones along with the substrate differences listed above. Conditions in the flat area could also have affected the stones so intensely that they were not suitable for habitation, likely due to threat of desiccation. All the un-colonized gravestones were not in the flat area, however 3 out of the 6 un-colonized marble gravestones were located in this area and the only un-colonized limestone gravestone. Since *O. anomalum* colonization showed a particular affinity for limestone, both within this study and in natural habitats, the un-colonized limestone gravestone sampled was an obvious and questionprovoking outlier. However, despite site differences that may have had their own influences on colonization, both level and sloping areas were necessary for a complete view of the cemetery so neither could be excluded. The unexpected results within sites could be explained by inhospitable conditions due to variables not tested in this study, but two alternative ideas discussed in other studies were dispersal and moss-lichen interactions.

Dispersal ability has been considered as a reason for deviations from the expected distribution patterns of moss species, in this case colonization of calcareous surfaces (Cleavitt 2001; Pharo and Beattie 2002; Kimmerer and Driscoll 2000). Most moss species are small and reside well within the boundary layer of their substrate for self-protection, the main exception to this is the extension of the sporophyte into the turbulent air currents above this zone. The benefit of this elevation is that some of the spores will escape the boundary layer and find an ideal habitat away from the parent plant. Most spores however end up only a short distance from their release site and even though some escape, other hardships await. Movement of a species to a new area depends on its propagules' ability to survive long enough to find a suitable habitat (Cleavitt 2002). Spores for different species have different physiologies; they range in size, surface features and longevity. If a spore does not find a habitat with the right complement of biotic and abiotic factors to support its growth and development within a certain time period it will effectively lose its propagation ability. Asexual propagules are also most often dispersed within a short distance from the parent plant, where they could form new colonies or assist in the formation of a colonial mat, but even in this situation growth is not always assured. Cleavitt in her study on substrate physiology found a discrepancy between vegetative propagule physiology and adult habitat conditions, even though conditions were suitable for the adult the propagules were unable to establish or were doing so at a very low rate. This low rate of establishment suggests that even seemingly ideal areas already proven suitable for the species could have colonization barriers (2002). Though spore production can be high, establishment of spores and mature plant development is low leading to a greater dependency on this vegetative growth via asexual propagules and colonial spread for establishment (Kimmerer and Driscoll 2000). However, even these asexual propagules must survive the harsh environment outside the boundary layer to move to a new area.

Kimmerer and Driscoll also mention dispersal capacity, but couple this with habitat density and distance between patches as important factors to persistence of bryophyte communities, since all these factors influence migration and extinction processes (2000). If colonization is impeded due to barriers to air currents which could carry spores and asexual propagules, such as vegetative growth and stone placement around a site, it could explain why an otherwise ideal location is un-colonized or only has a small population. For instance the colonized limestone stone with the smallest percent moss cover was protected almost completely on all sides by grave markers and vegetation which could explain in part the reduced establishment of spores and fragments. Determining whether this site does receive fewer propagules would require further study.

Organism distribution theories and models have also been applied to moss colonization to determine if the differences in moss colonization on suitable sites are related to dispersal. To test whether moss composition follows the theory of island biogeography, Kimmerer and Driscoll did a study on insular boulders and found moss composition did not support this theory (2000). An alternate hypothesis put forth is that moss species more closely resemble the metapopulation model which means the distribution and number of source populations becomes important.

Little is known about the interaction and potential competition between lichen and bryophytes living on the same substrate. Tree studies have shown that moss and lichen species inhabit different vertical strata, which is a pattern that may translate to stone environments (Gabriel and Bates 2005). Many of the lichens observed inhabited the upper sections of stones while *O. anomalum* colonization favored the bottom, though there was some joint colonization and overtopping by lichen. This pattern may also relate to greater exposure to wind and sunlight with upward vertical movement, i.e. none of the spires were colonized by *O. anomalum* regardless of rock type or coverage on lower parts of the stone. Pharo and Beattie in a study of bryophyte and lichen diversity found fewer bryophytes in drier areas which may be why lichen is more common in the flat plot which has a greater incidence of sunlight and wind (2002). Whether the lichen species will crowd out the moss species is uncertain, though the one un-colonized limestone gravestone was extensively covered in several species of lichen. The lack of moss colonization could also involve previous colonization by lichen and occupation of ideal *O. anomalum* niche spaces. Lichen species are capable of producing many unique compounds which discourage plant growth. These substances and others that help them break down the substrate for nutrient purposes may have created an unfavorable environment for *O. anomalum* colonization (Brodo et al. 2001). Further studies on the relationship between these groups may prove interesting.

This study was primarily to determine distribution patterns of *O. anomalum* within Oakwood Cemetery and what characteristics had the greatest influence on its distribution pattern. However the study served a secondary purpose in determining whether *O. anomalum* would colonize non-calcareous rocks and found that colonization is possible. The granite stone with the highest moss percent cover was small in size, close to the ground and had carved letters, that may have caught propagules, facing in a northern direction. As it was also located in the flat plot with full sunlight exposure and no protection from the wind these characteristics are of great benefit and could explain in part why it was an exception. PH and mineral testing were not done for this stone so it is not known if it is a chemical anomaly for rock type.

Microtopography:

This study did not support a linear relationship between percent moss cover and microtopographic complexity. This could have been due to the sampling of only one moss species instead of a community of several species. If this study used more than one moss species without differentiating between the species, it would more likely find a linear relationship because the different species would more completely cover the stone due to reduced competition and niche partitioning. Different species specialize in colonization of different microtopographic features; for example mesic species would more probably colonize a concave surface while xeric species would find a convex surface suitable. These differences due to physiological adaptations could result in more species that are specialized to fill the different niches available on a more microtopographically complex surface. These differences could result in greater moss coverage as different species would have different limitations and while some would be limited to flat surfaces other may prefer vertical, an otherwise un-utilized niche. At present this is just a speculation, it would have to be tested. One species would most probably find conditions in one or a few microtopographic features more conducive to productivity and reproduction and preferentially colonize these spaces. Preference for microtopographic class was tested in this study as an alternative to a linear relationship between percent moss coverage and microtopographic complexity.

Since microtopographic complexity did not follow a linear pattern, the descriptive characteristic form was used to determine if there were differences in distribution based on microtopographic class on a finer scale. Four distinct microtopographic classes were used to determine if *O. anomalum* colonization followed a pattern of preference for a certain class, these were: horizontal, vertical, slant and curved. Though the microtopographic features sampled in this study were simplified in comparison to natural rock formations, they were still distinctive enough to be significantly different in moss percent cover among the microtopographic classes. This study found a greater moss coverage on horizontal and slanted surfaces than on curved and vertical surfaces. This result is in contrast to a study by Alpert on saxicolous assemblages where he found more moss coverage on steep surfaces (1986). However the Alpert study was done in a natural setting which did not have the flat simplified surfaces of this study and with a community of plants which included vascular species that add another level of competition. Discrepancies in position selection in this study may involve lack of competition with vascular plants, since stones with extensive vascular plant growth were excluded from sampling. Without competition, the horizontal and slanted surfaces are more conducive to colonial mat formation since fewer colonies are lost to gravity and rainfall. Though the relationship between vascular plant competition and the stress tolerance of mosses favors avoidance as a strategy for moss persistence, *O. anomalum* is not competing in these study sites so can colonize any beneficial surface type. This dynamic may change if Oakwood Cemetery is left to develop into a more natural setting, but at present it is impeded by human maintenance of the current state. Alpert did however agree with this study that microtopographic class had a significant influence on moss distribution (1986).

The same Alpert study also disagreed with the results of this study about aspect, though the significant difference was based on an assemblage rather than a single species; this also may be the result of my site being wooded at various levels compared to the consistent open area of Alpert's site (1986). The north and east sides in this study did have higher mean percent cover which followed observed but without statistical significance as expected from this observation. Alpert related this separation to solar radiation which varies in my plot sites (1986). Another study also found that the influence of aspect can change based on the state of surrounding conditions (Pharo and Beattie 2002).

Age:

This study was unable to establish a relationship between age of the gravestones and percent cover of *O. anomalum.* Even though Oakwood Cemetery was established at a known time and gravestone age can be estimated, there is no absolute way to determine when stones were colonized. As mentioned above colonization is related to dispersal distance and mechanisms and once spores escape the boundary layer turbulent wind currents could sweep them in any direction. Other issues could have influenced the presence of *O. anomalum*, for instance colony removal. Though I was careful not to dislodge any colonies from the stones sampled, previous observation and the remnants found on the ground around stone sites have indicated a tendency for *O. anomalum* to be displaced. Since the cemetery is a well trafficked area, sites could have been disturbed prior to sampling or stones could have been cleared for maintenance purposes. This would have a major influence on this relationship by effectively restarting the colonization and establishment clock.

Further Considerations:

This study was designed to learn more about the distribution patterns of one species. Many of the studies prior to this one have focused on communities of species. Consequently much of the data used as comparison in this paper was about communities. However since communities can be divided into several single species, I feel that the information gathered still applies to this study.

The second concern is about where this study fits within the bryology field and its relevance for future studies including those within natural systems. Whether natural systems would have the same coverage is unknown but this study does support the same specificity for calcareous rocks (Crum 2004). This brings up the rather interesting question of what other species, even vascular species, could use ecological analogs in the form of anthropogenic habitats. In an increasingly human transformed world alternative environments for valuable plant species could become increasingly important. As far as relatedness to moss distribution within a natural environment, many of the same conditions for gravestones within a cemetery setting apply to rock outcrops and boulders. This setting just simplified the field collection component and allowed me to focus on a single species without the complication of isolating it from a group. This also allowed me to see the distribution of *O. anomlam* without natural constraints such as vegetative growth which is hard to orchestrate in a natural setting for most species.

Conclusion:

The distribution of bryophytes is influenced by a complex relationship between the biotic and abiotic components of their environment. These environmental characteristics are variable even in constructed settings. This study focused on substrate, microtopography and age and how these characteristics influenced the colonization of the saxicolous moss *Orthotrichum anomalum* in the anthropogenic habitat of gravestones. This study found a high level of substrate specificity, which was consistent with the natural distribution pattern of *O. anomalum* on calcareous substrates, by its preferential colonization on marble and limestone gravestones. Microtopographic classes were also found to have a significant influence on *O. anomalum* distribution. The lack of relationship for microtopographic complexity, coupled with the significance of the classes, suggests that fine scale differences in microtopography are more important as indicators of *O. anomalum* distribution in this anthropogenic habitat. This study was unable to find a significant relationship between estimated age and *O. anomalum* cover within the study site.

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