POLLUTION SENSITIVITY OF MACROLICHENS ON SOUTHERN LIVE OAK (*QUERCUS VIRGINIANA*)

by

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Jon Moore, and has been approved by the members of her supervisory committee. It was submitted to the faculty of The Honors College and was accepted in partial fulfillment of the requirements for the degree of Bachelor of Science in Liberal Arts and Sciences.

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ABSTRACT

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Lichens in south Florida are poorly documented. I surveyed macrolichens on the bark of Southern live oak trees (*Quercus virginiana*) present on the John D. MacArthur campus of Florida Atlantic University in Jupiter, Florida, to establish a baseline and to determine how automobile pollution may impact lichen species diversity and density. Ten randomly-selected trees in each of two study areas (adjacent to Parkside Drive versus mid-campus, away from any road) were surveyed for lichen species diversity and coverage. Fifteen species of lichens were identified using identification keys and chemical spot tests. Lichen diversity was similar in the two areas of study, but overall lichen coverage was significantly lower in the area adjacent to Parkside Drive, likely due in part to greater exposure to air pollutants from automobile exhaust.

To the “unenlichened” who like to liken lichens to plants

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

Lichens are a symbiotic association between one or more mycobionts (fungi) and one or more photobionts, such as cyanobacteria or algae (Purvis 2000; Spribille et al. 2016). The fungi provide the main structural component of the lichen, while the photobionts photosynthesize to produce carbohydrates, in addition to providing nitrogen compounds (Richardson 1992). Lichenized fungi, or the species of fungi that are engaged in a symbiotic relationship with a photobiont, differ from non-lichenized fungi because they go through a morphogenesis in which the photobiont activates certain fungal genes to result in a unique structure and appearance (Brodo et al. 2001; Nash 2008). Lichens are classified based on the primary species of lichenized fungus that they contain. Photomorphs are lichens that include the same fungus species but different photobionts (Purvis 2000).

The entire structural body of the lichen is known as the thallus (plural: thalli). Most of the cells of a lichen are fungal cells in the form of threads known as hyphae. In addition, lichens have a single layer of photobiont cells (Brodo et al. 2001). Basic lichen anatomy includes the cortex, which is the protective outside layer of fungal cells, with the photobiont layer of algal cells below, and then the medulla, a thick and often white layer composed of hyphae (Brodo et al. 2001). Characteristics used to identify different lichen species include cilia (hair-like growths at the edges of the thallus), maculae (white patches visible on the surface from a natural break in the photobiont layer), and pseudocyphellae (bumps on the surface as a result of hyphae growing through a break in the cortex) (Brodo et al. 2001). Different lichens produce unique secondary metabolites, or the by-product compounds of metabolism (Brodo et al. 2001). The metabolites react with chemicals to give different colors, so spot tests in which a small drop of chemical is applied to the cortex or medulla of a lichen are used to distinguish between species (Brodo et al. 2001). Reproductive structures of lichens are also used to differentiate species. Four structures commonly used for identification are pycnidia (structures that contain spores that appear as black dots; Figure 1), apothecia (cup-shaped fruiting bodies that contain spores; Figure 1), soredia (clumps of fungal cells surrounding algal cells; Figure 2), and isidia (finger-like outgrowths of the cortex) (Brodo et al. 2001).

Figure 1. *Parmotrema subrigidum* with pycnidia (black dots) and apothecia (arrow).

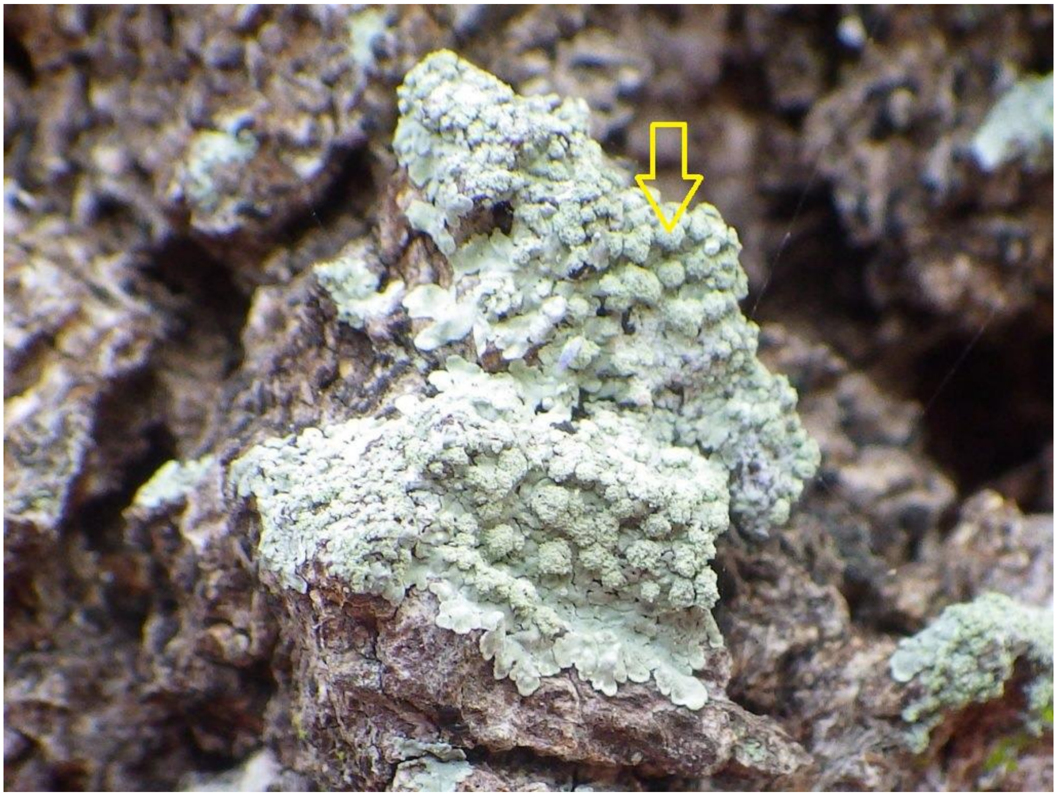

Figure 2. *Dirinaria picta* with soralia composed of clumps of soredia (arrow).

In addition to their taxonomic classifications, lichens are generally categorized into one of three major types based on their physical structure: fruticose, foliose, and crustose (Hale 1969). The term macrolichens refers to fruticose and foliose lichens because their thalli have a greater surface area that is not as firmly attached to their substrates compared to the thalli of the crustose lichens (Brodo et al. 2001). Macrolichens also have a lower cortex or cortical layer on the side that attaches to the substrate, whereas crustose lichens do not have a lower cortical layer and attach via their medulla layer (Brodo et al. 2001).

It is particularly difficult to distinguish between different crustose species due to their similarities in appearance. Identification often requires additional chemical tests using techniques such as thin layer chromatography or spore analysis. Therefore, I narrowed the scope of this project to macrolichen species, identified through the use of keys and simple chemical spot tests. A fourth type of lichen, squamulose, is characterized by a mixture of traits associated with crustose and foliose lichens. Squamulose lichens differ from foliose lichens in that they do not have a lower cortex, but they are also not as firmly attached to their substrate as crustose lichens, so very few species fall into the squamulose category (Hale 1969). However, some foliose species appear squamulose, so the classification is relevant to the current research.

Lichens play multiple, important ecological roles, including the creation of soil in primary succession, which allows for the growth of plants and all other species that rely on producers (Nash 2008). They are present in most terrestrial habitats all over the world as a result of their ability to tolerate a wide range of temperatures and environmental conditions (Nash 2008). To satisfy their hydration needs, lichens absorb water from dew, rain, and humidity. They grow very slowly, from one to five millimeters per year on average, due in part to the fact that they undergo metabolism and photosynthesis only when moist and become inactive when dry (Richardson 1992).

Automobile exhaust from internal combustion engines is a major source of air pollution in urban areas. A few examples of primary air pollutants found in car exhaust include nitrogen oxides, sulfur dioxide, soot, and carbon monoxide (Richardson 1992). Sulfur and nitrogen compounds react with oxygen and water vapor to give sulfuric and nitric acids, which are main components of acid rain and industrial smog. Dissolved sulfur dioxide and particulates get absorbed and accumulated by lichens, disrupting their metabolic processes and damaging chloroplasts and mitochondria (Richardson 1992). Consequently, lichens tend to be more sensitive to air pollution than larger plants and animals (Brodo 1966). Macrolichens are particularly sensitive to airborne pollutants because much of the thallus is exposed to the air (Cameron et al. 2007). Researchers take advantage of lichens' tendency to accumulate air pollutants by using them as a low-cost alternative to expensive techniques to measure air pollution (Nash 1973; Richardson 1992; Asta et al. 2002; Cameron et al. 2007; Will-Wolf et al. 2017).

The objectives of my research are to create a baseline of the species of macrolichens on the bark of Southern live oak trees (*Quercus virginiana*) that are present on the John D. MacArthur campus of Florida Atlantic University in Jupiter, Florida, and to compare species coverage between two areas of study in varying proximity to a major road to determine how automobile pollution impacts lichen density and diversity. Relatively little research has been conducted on lichens in southeastern Florida, except in the Everglades region (Moore 1968; Harris 1995; Kaminsky 2011; Seavey and Seavey 2011, 2012, 2014; Lücking et al. 2011), so my project aims to document the species that occur on campus and to collect data on coverage of different classifications of lichens. Such identification and coverage data will create a baseline, or the necessary starting point, from which future studies can be designed on topics such as changes in biodiversity and lichen density over time or in response to anthropogenic alterations to the environment.

Site Description

The project takes place in two areas of study in varying proximity to Parkside Drive, a major thoroughfare that borders the west side of the John D. MacArthur campus (hereafter referred to as campus) (Figure 3).

Figure 3. Areas of study on the John D. MacArthur campus.

The first area of study (AOS1) is in the central part of campus, in the corridor between the Honors College (MC01), Administration (MC02), and Student Resources (MC03) buildings (Figure 4).

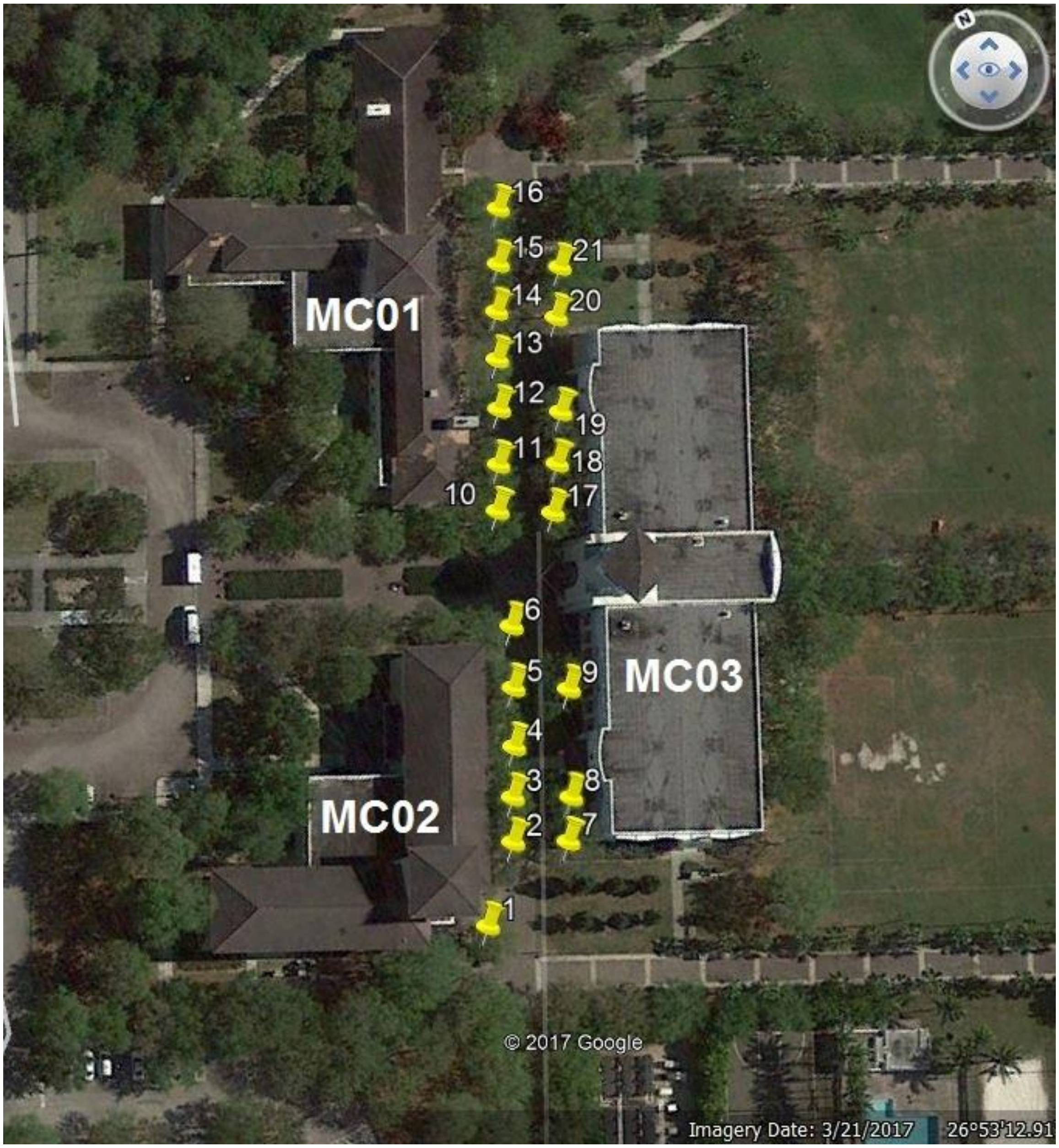

Figure 4. Area of study one (AOS1) with live oak trees (*Quercus virginiana*) labelled.

The second area of study (AOS2) is adjacent to Parkside Drive, a main thoroughfare that runs north from Donald Ross Road (Figure 5).

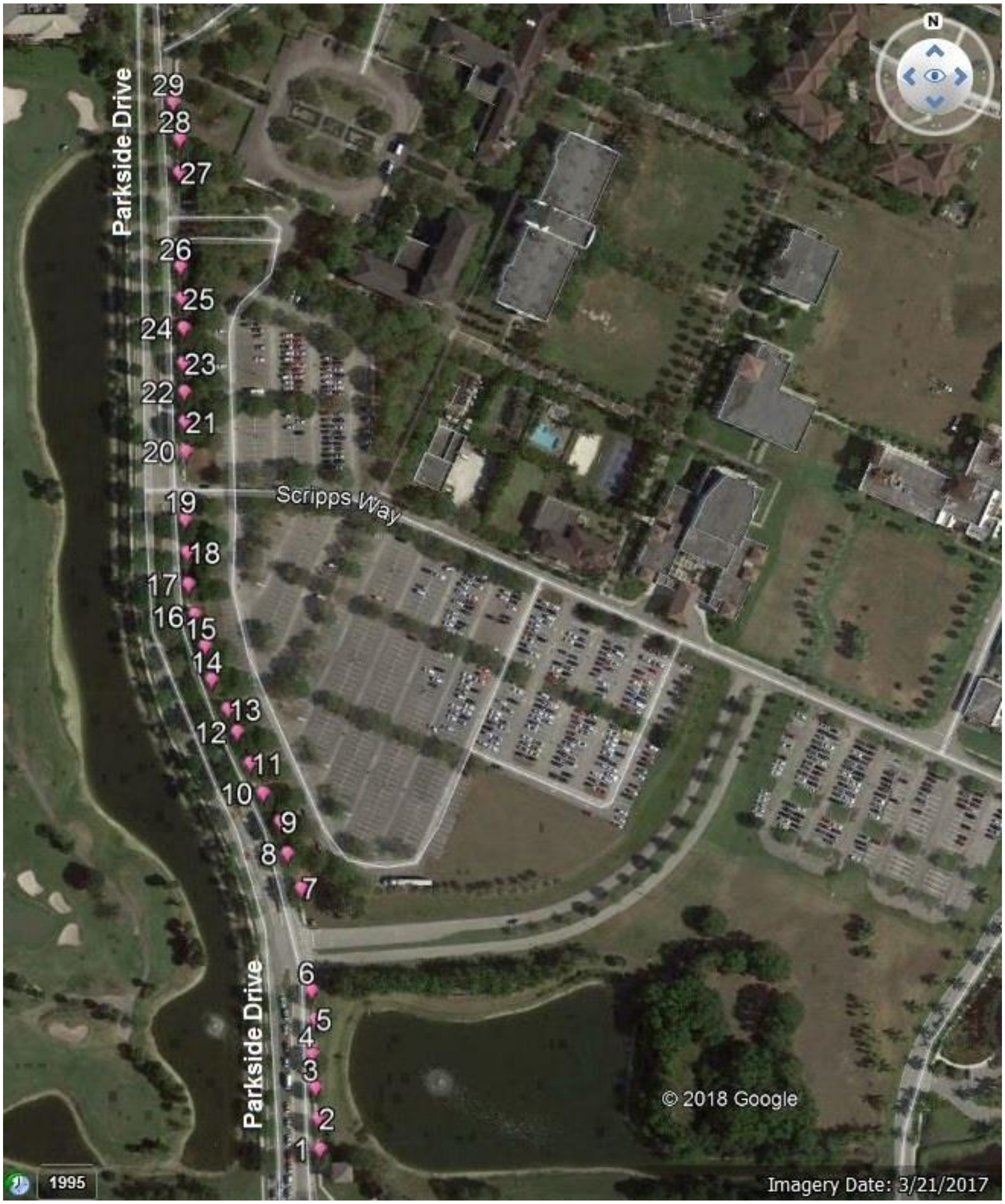

Figure 5. Area of study two (AOS2) with live oak trees (*Quercus virginiana*) labelled.

To minimize complicating factors such as variations in bark acidity for different species of tree and various ages of trees, one species of tree (Southern live oak) was chosen, and the trees selected were all planted in the same year. Based on an analysis of aerial maps of the campus and surrounding development in the years that it was first built, two areas of study were designated as containing Southern live oak trees that were planted around the same time, i.e. 1999 (aerial photos from original Abacoa Development Company). Southern live oak trees were identified and assigned a number from 1 to 21 in the first area of study and 1 to 29 in the second area of study. Ten sample trees in each area of study were designated using an online random number generator (random.org). Duplicate numbers were excluded, so numbers were generated until there were ten different sample trees designated (Table 1).

Table 1. Designated sample tree numbers

Sample trees from area of study 1	**Sample trees from area of study 2**
2, 4, 5, 7, 9, 10, 11, 15, 17, 18	4, 7, 8, 11, 12, 13, 14, 19, 25, 29

Data Collection

One transparency sheet with a 10 cm² quadrat printed on it along with three colors of water soluble markers (blue, green, and red) were used to trace different types of lichens for coverage to be calculated (Figure 6). The red marker was used to trace fruticose lichens, the green marker was used to trace foliose lichens, and the blue marker was used to trace what I described as squamulose lichens, which were later identified as small-lobed foliose lichens. Each quadrat was assigned a label indicating the sample tree number, the cardinal direction, and vertical position of the quadrat, with 1 being the highest quadrat in a given direction and 3 being the lowest (e.g., T2N1 refers to the first quadrat on the north side of sample tree 2).

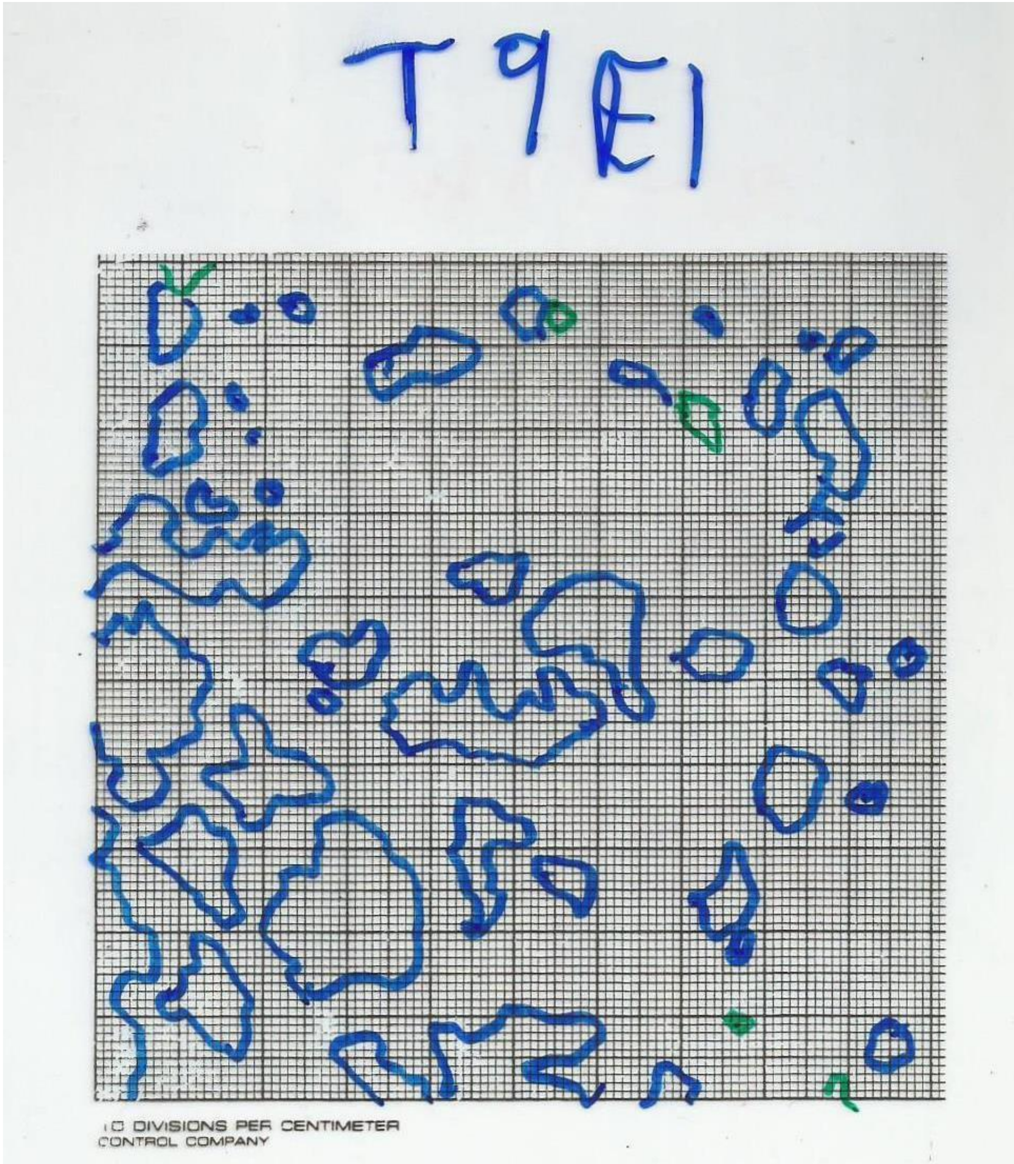

Figure 6. First quadrat on the east side of sample tree nine in AOS1.

The first quadrat was attached to the trunk of each mature tree about 1.5 m above the ground using four thumb tacks. The next quadrat was 20 cm lower, and the third quadrat, 20 cm below the second. Thus, there were 10 cm between each of the three quadrats in a given cardinal direction in vertical alignment for a total of twelve quadrats per sample tree. The height of the quadrats was chosen to avoid the influence of the sprinkler irrigation system, which may artificially stimulate lichen growth at the base of the trunk.

To calculate the coverage of the different types of lichen within the quadrat, the transparency sheet was scanned for the samples in AOS1, but photographed for the samples in AOS2 due to the greater distance I needed to travel from the site to my computer scanner. Images of the transparency sheets were then uploaded to an online irregular shape area calculator (sketchandcalc.com) to determine the coverage area (mm^2) of each type of lichen (fruticose, foliose, and squamulose) within each quadrat.

Fruticose and foliose specimens were collected from each of the ten sample trees in the two areas of study for identification. To prevent the removal of the only specimen of a given species from a sample tree, photographs were often taken of the fruticose species and sometimes taken of the foliose species for identification rather than collecting a specimen. Small segments of a solitary specimen were occasionally collected to conduct spot tests if the species could not be identified using the photographs alone. The identification keys from *Lichens of North America* and "Field oriented keys to the Florida lichens" were the primary keys used to identify specimens (Brodo et al. 2001; Rosentreter et al. 2015). Spot tests consisted of the application of a single drop of 10% potassium hydroxide (abbreviated as K), sodium hypochlorite solution or bleach (abbreviated as C), or *para*-phenylenediamine solution (abbreviated as PD) using drawn-out capillary tubes under a dissecting microscope (detailed descriptions on how to make chemical solutions found in Brodo et al. 2001).

Data Analysis

After uploading the scans or photographs of the quadrats to the online area calculator tool to determine coverage area (mm^2) of each type of lichen, data were recorded in a Microsoft Excel spreadsheet for analysis (Appendix I and II). Total lichen coverage (mm^2) was calculated for each quadrat by summing the coverage for each of the three lichen types. A total of twenty-one Student's t-tests were conducted on various subsets of data.

Identification

Identification of species of lichens started with the assignment of a specimen to one of four categories (crustose, foliose, squamulose, and fruticose) in the field while coverage data were collected. The lichens that were identified as crustose species were excluded from the coverage calculations as beyond the scope of the project (not macrolichens). The lichens that were described as foliose and marked with a green marker in the quadrats included the seven *Parmotrema* species that were later identified with spot tests when specimens were collected. The lichens that were identified as squamulose and marked with a blue marker to distinguish them from the foliose lichens included the three *Dirinaria* species, which were later identified as foliose as well. For the purposes of this project, they are described as squamulose, but it should be noted that very few lichen species fall into the squamulose category and that *Dirinaria* species are recognized as small-lobed foliose in identification keys. The lichens that were identified as fruticose and marked with a red marker included the five *Ramalina* species.

Once the squamulose specimens were identified as belonging to the genus *Dirinaria*, physical characteristics were sufficient for distinguishing between species using the descriptions found in the identification keys of *Lichens of North America*. The specimens of *D. picta* are characterized by soredia, the asexual reproductive structures previously described and depicted in Figure 2. The *D. confusa* and *D. purpurascens* specimens have black and purple apothecia, respectively.

The foliose lichen *Parmotrema praesorediosum* produces no color change in reactions with the medulla during spot tests because of the presence of caperatic acid (Brodo et al. 2001). Therefore, when the three spot test reactions with potassium hydroxide (K), bleach (C), and *para*-phenylenediamine (PD) were negative for color changes, the species was identified as *P. praesorediosum.* The color of the underside of the specimen and the presence of cilia, pycnidia, and/or maculae initiated spot tests to distinguish between species that have similar sets of physical characteristics. A few of the physical differences between the specimens of *P. praesorediosum* and *P. tinctorum* were the presence of soredia and a mottled ivory underside (*P. praesorediosum*) versus isidia and a brown underside (*P. tinctorum*), as well as a positive reaction to bleach, which turned the medulla red. An example of physical differences among specimens of *P. praesorediosum*, *P. hypoleucinum*, and *P. subrigidum* was absence of cilia (*P. praesorediosum*) versus presence of cilia (*P. hypoleucinum* and *P. subrigidum*). The specimens of *P. perforatum* and *P. subrigidum* differed from *P. praesorediosum* by the presence of apothecia rather than soredia.

Many of the acids that the fruticose species contain give negative spot test results for the chemicals that were used, so identification of fruticose species relied heavily on analysis of photographs and observation of specimens under a dissecting microscope. The physical differences used to differentiate amongst fruticose species include patterns of pseudocyphellae, the presence or absence of apothecia, and the size and shape of branches. The most evident physical difference between specimens of *R. complanata* and those of *R. stenospora* was the number and shape of pseudocyphellae, which were abundant on thicker branches for the former species and elongated on narrower branches for the latter species. Identification using published keys was conducted as accurately as possible, but the lack of prior experience and training on lichen identification may have resulted in some incorrect identifications.

RESULTS

Identification

Fifteen different foliose and fruticose lichen species were identified using keys and a combination of photographs and chemical spot tests on collected specimens. Five species were only found in the first area of study and four species were only found in the second area of study (Table 2). I photographed all identified species except *Parmotrema hypoleucinum* (Appendix III).

The most commonly identified species of foliose lichen was *Parmotrema praesorediosum*, while the most commonly identified fruticose lichen species was *Ramalina complanata*, followed closely by *R. stenospora*. A single specimen was identified for each of the two species *R. peruviana* and *R.* cf. *americana*, both in the second area of study adjacent to Parkside Drive.

Table 2. Locations of identified foliose and fruticose lichen species

Species found in both areas	**AOS1 Species**	**AOS2 Species**
Dirinaria confusa	*Dirinaria purpurascens*	*Parmotrema crisitferum*
Dirinaria picta	*Parmotrema hypoleucinum*	*Parmotrema tinctorum*
Parmotrema praesorediosum	*Parmotrema perforatum*	*Ramalina* cf. *americana*
Parmotrema subrigidum	*Parmotrema reticulatum*	*Ramalina peruviana*
Ramalina complanata	*Ramalina denticulata*	
Ramalina stenospora		

Pollution impacts

Mean lichen coverage for the three documented types of lichens was 1937 mm^2 (SD = 1549 mm^2) for AOS1 (n = 120) and 1227 mm^2 (SD = 1538 mm^2) for AOS2 (n = 120). I calculated individual, total, and mean coverage for each cardinal direction in the two areas of study (Table 3 and 4).

Table 3. Area of study 1 quadrat coverage (mm^2) by direction

Tree	Quadrat	North	South	East	West
2	1	933	233	32	757
2	2	209	772	0	1241
2	3	577	215	719	2069
4	1	1620	2071	317	1890
4	2	1661	1909	106	4593
4	3	676	3842	323	4761
5	1	1708	635	2635	1356
5	2	1573	475	2565	1522
5	3	2588	1599	1077	3180
7	1	916	747	141	2113
7	2	1124	2071	1188	1863
7	3	1895	1602	391	3976
9	1	1247	3464	2829	537
9	2	2122	717	224	219
9	3	1667	1563	790	524
10	1	2273	4280	3576	849
10	2	3706	4794	4443	6753
10	3	5539	3144	4060	5128
11	1	5808	1425	2235	1879
11	2	5656	1606	2318	2839
11	3	3977	3896	1248	2110
15	1	910	3020	4512	908
15	2	365	2241	5012	645
15	3	425	1909	4596	403
17	1	4404	1576	2943	599
17	2	3623	1569	1583	2186
17	3	1890	1507	1907	696
18	1	2362	180	0	214
18	2	2885	603	292	165
18	3	3858	378	1813	374
Total coverage:		68197	54043	53875	56349
Average coverage:		2273	1801	1796	1878
Maximum:		5808	4794	5012	6753
Minimum:		209	180	0	165

Table 4. Area of study 2 quadrat coverage (mm^2) by direction

Tree	**Quadrat**	**North**	**South**	**East**	**West**
4	1	3407	1099	1769	1782
4	2	3497	1291	1825	1661
4	3	2424	4386	315	2100
7	1	4515	1888	1102	2491
7	2	1483	652	506	4368
7	3	1180	652	614	1496
8	1	781	90	0	489
8	2	912	267	106	846
8	3	621	158	21	79
11	1	162	355	0	807
11	2	1236	712	0	523
11	3	1885	1598	0	814
12	1	108	85	14	596
12	2	157	34	0	1065
12	3	301	186	162	561
13	1	364	25	177	634
13	2	1389	123	503	1468
13	3	2167	758	2780	3196
14	1	68	0	0	63
14	2	184	12	413	817
14	3	1055	38	404	341
19	1	587	2906	114	377
19	2	1536	4416	1396	537
19	3	1282	7322	1510	3360
25	1	6516	369	174	158
25	2	6176	2751	644	3510
25	3	6406	2236	2117	5335
29	1	67	198	256	197
29	2	263	200	773	144
29	3	2532	271	371	1065
Total coverage:		53261	35078	18066	40880
Average coverage:		1775	1169	602	1363
Maximum:		6516	7322	2780	5335
Minimum:		67	0	0	63

Of the seventeen t-tests comparing general lichen coverage in quadrats facing different directions, three were significant at the α = .01 level and four were significant at the α = .05 level (Table 5).

Table 5. Student's t-test p-values for directions within and between areas of study

Area of study 1		**Area of study 2**		**Both areas of study**	
North and south	0.217	North and south	0.200	North	0.282
East and west	0.847	East and west	0.010	East	0.001
North and east	0.256	North and east	0.003	South	0.110
South and west	0.843	South and west	0.629	West	0.198
North and west	0.358	North and west	0.341	Overall sum	0.0004
East and south	0.988	East and south	0.102		

Of the four t-tests comparing lichen coverage between the two areas of study by lichen type, one was significant at the α = .01 level and two were significant at the α = .05 level (Table 6).

Table 6. Student's t-test p-values for lichen types between areas of study

Both areas of study		**AOS1 mean coverage (mm^2)**	**AOS2 mean coverage (mm^2)**
Foliose	0.075	1174	833
Fruticose	0.052	74	34
Squamulose	0.016	1006	735
Foliose and squamulose	0.002		

DISCUSSION

Previous studies show that fruticose and foliose lichen species have lower tolerances for air pollution, and macrolichen coverage and diversity tends to increase on a gradient as one travels away from areas with high concentrations of sulfur dioxide and other air pollutants (Brodo 1966; Nash 1973; Richardson 1992; Cameron et al. 2007). Species absent from the second area of study could be indicator species with a lower tolerance for air pollution: *D. purpurascens*, *P. hypoleucinum*, *P. perforatum*, *P. reticulatum*, and *R. denticulata*. Contrary to expectations, only one of the five species identified from the first area of study that was not identified in the second area of study was fruticose, while two species of fruticose lichens, *R.* cf. *americana* and *R. peruviana*, were identified from the second area of study but not the first.

One possible explanation for such results is that species that were not identified in one area of study or another are not necessarily absent from that area, but instead they were left unidentified due to specimen collection being restricted to sample trees and the non-comprehensive nature of the study. On the other hand, the results may indicate that fruticose species such as *R. complanata* and *R. stenospora* are present in both areas of study because they can tolerate pollution levels adjacent to the road to an extent. Although the Student's t-test comparing fruticose coverage in both areas of study was not significant at the $\alpha = .01$ level, the results were close to being statistically significant at the $\alpha = .05$ level with a value of 0.052, indicating that the likelihood of the two groups showing differences in coverage due to chance is lower than that of many of the other groups that were tested. The mean coverage of each of the three types of lichens for the quadrats in which they were present in the first area of study, which was farther from the road, was greater than the mean coverage of each of the lichen types in the second area of study (Table 6).

Species belonging to the fruticose lichen genus *Usnea* are common in Florida but were not identified in either area of study. Species of *Usnea* have thin branches with a comparatively large surface area exposed, so their sensitivity to sulfur dioxide levels may preclude them from colonizing urbanized regions, which include both of the areas of study (Richardson 1992). Many of the previous studies on lichens and air quality, which recognize a decrease in coverage along a gradient, involved data collection in sites that were kilometers apart (Brodo 1966; Richardson 1992; Cameron et al. 2007). The current project's areas of study were located less than a kilometer apart, which may explain the similarity of species diversity between the two areas.

The common factor that returned significant results when comparing general lichen coverage was coverage on the east side of sample trees in the second area of study. In both areas of study, there was higher lichen concentration on the north and west sides than on the east and south sides of the trees, as shown by the total and mean coverage data for the four directions in Tables 3 and 4. Unlike vascular plants, lichens do not have a mechanism to retain water, and they rely on precipitation to satisfy their hydration needs, as previously mentioned. Because they undergo metabolism and growth only when hydrated, lichens that are exposed to higher temperatures dry out more quickly and grow more slowly (Nash 2008). The east and south sides of the trees therefore have less lichen coverage because they are subjected to more sunlight, which dries out the lichens, making those orientations of the tree less habitable. Despite the west side of the trees more directly facing the source of air pollution from the passing traffic, the east side of the trees in the second area of study contained the least amount of lichen coverage due to the physiology of the lichens in relation to sun exposure.

In comparing the two areas of study in terms of the coverage of individual lichen types, the results of the t-tests showed that the tests comparing the species that were designated as squamulose and the species that were designated as foliose and squamulose together were significant. As previously mentioned, the squamulose designation included the three species that were later identified as *Dirinaria confusa, D. picta, and D. purpurascens*, which are three species of foliose lichens that have smaller lobes and therefore resemble crustose lichens. Upon testing of the overall foliose coverage, which included the species that were designated as foliose and squamulose that were later identified as *Parmotrema* and *Dirinaria* species, the results are significant at the $\alpha = .01$ level, indicating that it is not likely that the difference in lichen coverage between the two areas of study is due to chance (Table 6). The results show that the difference in foliose coverage between the two areas was statistically significant while the difference in fruticose coverage was not, possibly signifying that that the foliose species that were present in the two areas of study exhibit greater sensitivity to air pollution between shorter distances away from the source.

The result of the Student's t-test that aimed to compare overall lichen coverage between the two areas of study produced significant results that clearly show that there is a difference that is probably caused in large part by proximity to a source of air pollution (Table 5). In addition to air pollution, another factor that may explain the difference in lichen density between the two areas is the amount of shade versus sun exposure based on the canopy size of the trees and proximity to buildings as an additional source of shade. Further research on concentrations of sulfur compounds in lichen specimens and the identification and collection of coverage data for crustose lichens may contribute to a greater understanding of lichen sensitivity to air pollution.

CONCLUSION

A baseline of lichen species that are present on campus was created through identification of specimens collected from each of ten sample trees in two areas of study for a total of twenty Southern live oak trees. The expected number of lichen species to be identified prior to conducting the study ranged from ten to twenty, so the final results in which fifteen species were identified and documented conformed to expectations.

The Student's t-tests that produced significant results at the $\alpha = .05$ level included the tests that compared general lichen coverage between the east and west sides and north and east sides of the trees in the second area of study next to the road, the east sides of the trees in both areas of study, and overall lichen coverage between the two areas of study on all sides of the trees. The tests that produced significant results at the $\alpha = .05$ level when comparing the coverage of individual lichen types included squamulose or *Dirinaria* species coverage in the two areas of study and both squamulose and foliose or *Dirinaria* and *Parmotrema* species coverage in the two areas of study.

Lichen diversity was similar between the two areas of study, with eleven species identified from the first area of study and ten species identified from the second area of study with six species in common. The number of species that were identified may not necessarily reflect the lichen diversity in the two areas of study, as specimen collection was limited to sample trees. According to the results, the amount of lichen diversity did not differ between the two areas of study, but overall lichen density differed significantly, probably in part due to proximity to air pollution from automobile exhaust.

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Appendix I. Area of Study 1 Coverage Data

Quadrat	**Lichen type**	**Marker color**	**mm²**	**Quadrat sum**
T2N1	foliose	green	632	
T2N1	squamulose	blue	301	933
T2N2	foliose	green	24	
T2N2	squamulose	blue	185	209
T2N3	foliose	green	239	
T2N3	squamulose	blue	338	577
T2E1	squamulose	blue	32	32
T2E2			0	0
T2E3	foliose	green	615	
T2E3	squamulose	blue	104	719
T2S1	squamulose	blue	233	233
T2S2	foliose	green	123	
T2S2	squamulose	blue	649	772
T2S3	foliose	green	62	
T2S3	squamulose	blue	153	215
T2W1	foliose	green	18	
T2W1	squamulose	blue	739	757
T2W2	foliose	green	1080	
T2W2	squamulose	blue	161	1241
T2W3	foliose	green	1479	
T2W3	squamulose	blue	590	2069
T4N1	foliose	green	507	
T4N1	squamulose	blue	1113	1620
T4N2	foliose	green	175	
T4N2	squamulose	blue	1486	1661
T4N3	squamulose	blue	676	676
T4E1	squamulose	blue	317	317
T4E2	fruticose	red	33	
T4E2	squamulose	blue	73	106
T4E3	fruticose	red	278	
T4E3	squamulose	blue	45	323
T4S1	foliose	green	140	
T4S1	squamulose	blue	1931	2071
T4S2	foliose	green	940	
T4S2	squamulose	blue	969	1909
T4S3	foliose	green	2447	

T4S3	squamulose	blue	1395	3842
T4W1	foliose	green	187	
T4W1	squamulose	blue	1703	1890
T4W2	foliose	green	3966	
T4W2	squamulose	blue	627	4593
T4W3	foliose	green	4699	
T4W3	squamulose	blue	62	4761
T5N1	foliose	green	1505	
T5N1	squamulose	blue	203	1708
T5N2	foliose	green	934	
T5N2	squamulose	blue	639	1573
T5N3	foliose	green	1653	
T5N3	squamulose	blue	935	2588
T5E1	foliose	green	2255	
T5E1	squamulose	blue	380	2635
T5E2	foliose	green	2119	
T5E2	squamulose	blue	446	2565
T5E3	foliose	green	471	
T5E3	squamulose	blue	606	1077
T5S1	foliose	green	446	
T5S1	squamulose	blue	189	635
T5S2	squamulose	blue	475	475
T5S3	fruticose	red	18	
T5S3	foliose	green	621	
T5S3	squamulose	blue	960	1599
T5W1	foliose	green	209	
T5W1	squamulose	blue	1147	1356
T5W2	foliose	green	201	
T5W2	squamulose	blue	1321	1522
T5W3	fruticose	red	19	
T5W3	foliose	green	714	
T5W3	squamulose	blue	2447	3180
T7N1	foliose	green	536	
T7N1	squamulose	blue	380	916
T7N2	foliose	green	980	
T7N2	squamulose	blue	144	1124
T7N3	foliose	green	109	
T7N3	squamulose	blue	1786	1895
T7E1	squamulose	blue	141	141

T7E2	squamulose	blue	1188	1188
T7E3	fruticose	red	129	
T7E3	squamulose	blue	262	391
T7S1	squamulose	blue	747	747
T7S2	fruticose	red	18	
T7S2	foliose	green	723	
T7S2	squamulose	blue	1330	2071
T7S3	foliose	green	50	
T7S3	squamulose	blue	1552	1602
T7W1	squamulose	blue	2113	2113
T7W2	squamulose	blue	1863	1863
T7W3	squamulose	blue	1943	3976
T9N1	foliose	green	418	
T9N1	squamulose	blue	829	1247
T9N2	foliose	green	1875	
T9N2	squamulose	blue	247	2122
T9N3	foliose	green	1506	
T9N3	squamulose	blue	161	1667
T9E1	foliose	green	58	
T9E1	squamulose	blue	2771	2829
T9E2	foliose	green	18	
T9E2	squamulose	blue	206	224
T9E3	foliose	green	43	
T9E3	squamulose	blue	747	790
T9S1	foliose	green	941	
T9S1	squamulose	blue	2523	3464
T9S2	foliose	green	318	
T9S2	squamulose	blue	399	717
T9S3	foliose	green	177	
T9S3	squamulose	blue	1386	1563
T9W1	foliose	green	41	
T9W1	squamulose	blue	496	537
T9W2	squamulose	blue	219	219
T9W3	foliose	green	247	
T9W3	squamulose	blue	277	524
T10N1	foliose	green	786	
T10N1	squamulose	blue	1487	2273
T10N2	foliose	green	3386	
T10N2	squamulose	blue	320	3706

T10N3	foliose	green	4279	
T10N3	squamulose	blue	1260	5539
T10E1	foliose	green	2722	
T10E1	squamulose	blue	854	3576
T10E2	foliose	green	3992	
T10E2	squamulose	blue	451	4443
T10E3	foliose	green	3427	
T10E3	squamulose	blue	633	4060
T10S1	foliose	green	2329	
T10S1	squamulose	blue	1951	4280
T10S2	foliose	green	965	
T10S2	squamulose	blue	3829	4794
T10S3	foliose	green	478	
T10S3	squamulose	blue	2666	3144
T10W1	foliose	green	550	
T10W1	squamulose	blue	299	849
T10W2	foliose	green	6294	
T10W2	squamulose	blue	459	6753
T10W3	foliose	green	3771	
T10W3	squamulose	blue	1357	5128
T11N1	foliose	green	3540	
T11N1	squamulose	blue	2268	5808
T11N2	foliose	green	4196	
T11N2	squamulose	blue	1460	5656
T11N3	fruticose	red	95	
T11N3	foliose	green	2072	
T11N3	squamulose	blue	1810	3977
T11E1	fruticose	red	139	
T11E1	foliose	green	676	
T11E1	squamulose	blue	1420	2235
T11E2	foliose	green	1923	
T11E2	squamulose	blue	395	2318
T11E3	foliose	green	865	
T11E3	squamulose	blue	383	1248
T11S1	foliose	green	149	
T11S1	squamulose	blue	1276	1425
T11S2	foliose	green	264	
T11S2	squamulose	blue	1342	1606
T11S3	foliose	green	1872	

T11S3	squamulose	blue	2024	3896
T11W1	foliose	green	1392	
T11W1	squamulose	blue	487	1879
T11W2	foliose	green	1007	
T11W2	squamulose	blue	1832	2839
T11W3	foliose	green	1071	
T11W3	squamulose	blue	1039	2110
T15N1	squamulose	blue	910	910
T15N2	foliose	green	87	
T15N2	squamulose	blue	278	365
T15N3	squamulose	blue	425	425
T15E1	foliose	green	2921	
T15E1	squamulose	blue	1591	4512
T15E2	foliose	green	3159	
T15E2	squamulose	blue	1853	5012
T15E3	foliose	green	1393	
T15E3	squamulose	blue	3203	4596
T15S1	foliose	green	656	
T15S1	squamulose	blue	2364	3020
T15S2	foliose	green	624	
T15S2	squamulose	blue	1617	2241
T15S3	foliose	green	861	
T15S3	squamulose	blue	1048	1909
T15W1	squamulose	blue	908	908
T15W2	squamulose	blue	645	645
T15W3	foliose	green	44	
T15W3	squamulose	blue	359	403
T17N1	fruticose	red	30	
T17N1	foliose	green	2478	
T17N1	squamulose	blue	1896	4404
T17N2	fruticose	red	37	
T17N2	foliose	green	2109	
T17N2	squamulose	blue	1477	3623
T17N3	foliose	green	856	
T17N3	squamulose	blue	1034	1890
T17E1	foliose	green	145	
T17E1	squamulose	blue	2798	2943
T17E2	foliose	green	198	
T17E2	squamulose	blue	1385	1583

T17E3	foliose	green	94	
T17E3	squamulose	blue	1813	1907
T17S1	fruticose	red	13	
T17S1	foliose	green	104	
T17S1	squamulose	blue	1459	1576
T17S2	foliose	green	383	
T17S2	squamulose	blue	1186	1569
T17S3	foliose	green	480	
T17S3	squamulose	blue	1027	1507
T17W1	foliose	green	64	
T17W1	squamulose	blue	535	599
T17W2	foliose	green	863	
T17W2	squamulose	blue	1323	2186
T17W3	foliose	green	28	
T17W3	squamulose	blue	668	696
T18N1	foliose	green	1652	
T18N1	squamulose	blue	710	2362
T18N2	fruticose	red	31	
T18N2	foliose	green	1567	
T18N2	squamulose	blue	1287	2885
T18N3	fruticose	red	147	
T18N3	foliose	green	1403	
T18N3	squamulose	blue	2308	3858
T18E1			0	0
T18E2	foliose	green	27	
T18E2	squamulose	blue	265	292
T18E3	fruticose	red	56	
T18E3	foliose	green	576	
T18E3	squamulose	blue	1181	1813
T18S1	squamulose	blue	180	180
T18S2	fruticose	red	54	
T18S2	squamulose	blue	549	603
T18S3	squamulose	blue	378	378
T18W1	fruticose	red	147	
T18W1	foliose	green	67	214
T18W2	squamulose	blue	165	165
T18W3	fruticose	red	11	
T18W3	foliose	green	145	
T18W3	squamulose	blue	218	374

Appendix II. Area of Study 2 Coverage Data

Quadrat	Lichen type	Marker color	mm^2	Quadrat sum
T4N1	foliose	green	2918	
T4N1	squamulose	blue	489	3407
T4N2	foliose	green	2925	
T4N2	squamulose	blue	572	3497
T4N3	foliose	green	709	
T4N3	squamulose	blue	1715	2424
T4E1	foliose	green	1103	
T4E1	squamulose	blue	666	1769
T4E2	fruticose	red	53	
T4E2	foliose	green	248	
T4E2	squamulose	blue	1524	1825
T4E3	squamulose	blue	315	315
T4S1	foliose	green	259	
T4S1	squamulose	blue	840	1099
T4S2	squamulose	blue	1291	1291
T4S3	squamulose	blue	4386	4386
T4W1	foliose	green	1009	
T4W1	squamulose	blue	773	1782
T4W2	foliose	green	1217	
T4W2	squamulose	blue	444	1661
T4W3	foliose	green	1502	
T4W3	squamulose	blue	598	2100
T7N1	foliose	green	3559	
T7N1	squamulose	blue	956	4515
T7N2	foliose	green	438	
T7N2	squamulose	blue	1045	1483
T7N3	foliose	green	842	
T7N3	squamulose	blue	338	1180
T7E1	foliose	green	162	
T7E1	squamulose	blue	940	1102
T7E2	foliose	green	143	
T7E2	squamulose	blue	363	506
T7E3	foliose	green	420	
T7E3	squamulose	blue	194	614
T7S1	foliose	green	1292	
T7S1	squamulose	blue	596	1888

T7S2	foliose	green	303	
T7S2	squamulose	blue	349	652
T7S3	foliose	green	386	
T7S3	squamulose	blue	266	652
T7W1	foliose	green	764	
T7W1	squamulose	blue	1727	2491
T7W2	foliose	green	3976	
T7W2	squamulose	blue	392	4368
T7W3	foliose	green	750	
T7W3	squamulose	blue	746	1496
T8N1	foliose	green	677	
T8N1	squamulose	blue	104	781
T8N2	foliose	green	554	
T8N2	squamulose	blue	358	912
T8N3	foliose	green	474	
T8N3	squamulose	blue	147	621
T8E1			0	0
T8E2	squamulose	blue	106	106
T8E3	squamulose	blue	21	21
T8S1	squamulose	blue	90	90
T8S2	squamulose	blue	267	267
T8S3	squamulose	blue	158	158
T8W1	foliose	green	212	
T8W1	squamulose	blue	277	489
T8W2	foliose	green	483	
T8W2	squamulose	blue	363	846
T8W3	squamulose	blue	79	79
T11N1	squamulose	blue	162	162
T11N2	fruticose	red	62	
T11N2	foliose	green	59	
T11N2	squamulose	blue	1115	1236
T11N3	foliose	green	786	
T11N3	squamulose	blue	1099	1885
T11E1			0	0
T11E2			0	0
T11E3			0	0
T11S1	foliose	green	176	
T11S1	squamulose	blue	179	355
T11S2	foliose	green	155	

T11S2	squamulose	blue	557	712
T11S3	fruticose	red	6	
T11S3	foliose	green	26	
T11S3	squamulose	blue	1566	1598
T11W1	squamulose	blue	807	807
T11W2	foliose	green	33	
T11W2	squamulose	blue	490	523
T11W3	squamulose	blue	814	814
T12N1	squamulose	blue	108	108
T12N2	squamulose	blue	157	157
T12N3	squamulose	blue	301	301
T12E1	squamulose	blue	14	14
T12E2			0	0
T12E3	squamulose	blue	162	162
T12S1	squamulose	blue	85	85
T12S2	squamulose	blue	34	34
T12S3	squamulose	blue	186	186
T12W1	squamulose	blue	596	596
T12W2	foliose	green	19	
T12W2	squamulose	blue	1046	1065
T12W3	squamulose	blue	561	561
T13N1	squamulose	blue	364	364
T13N2	foliose	green	15	
T13N2	squamulose	blue	1374	1389
T13N3	foliose	green	37	
T13N3	squamulose	blue	2130	2167
T13E1	squamulose	blue	177	177
T13E2	squamulose	blue	503	503
T13E3	foliose	green	110	
T13E3	squamulose	blue	2670	2780
T13S1	squamulose	blue	25	25
T13S2	squamulose	blue	123	123
T13S3	foliose	green	51	
T13S3	squamulose	blue	707	758
T13W1	squamulose	blue	634	634
T13W2	foliose	green	25	
T13W2	squamulose	blue	1443	1468
T13W3	fruticose	red	29	
T13W3	foliose	green	1704	

T13W3	squamulose	blue	1463	3196
T14N1	squamulose	blue	68	68
T14N2	squamulose	blue	184	184
T14N3	foliose	green	926	
T14N3	squamulose	blue	129	1055
T14E1			0	0
T14E2	squamulose	blue	413	413
T14E3	squamulose	blue	404	404
T14S1			0	0
T14S2	squamulose	blue	12	12
T14S3	squamulose	blue	38	38
T14W1	squamulose	blue	63	63
T14W2	foliose	green	11	817
T14W3	squamulose	blue	341	341
T19N1	squamulose	blue	587	587
T19N2	fruticose	red	23	
T19N2	foliose	green	40	
T19N2	squamulose	blue	1473	1536
T19N3	squamulose	blue	1282	1282
T19E1	squamulose	blue	114	114
T19E2	squamulose	blue	80	1396
T19E3	squamulose	blue	74	1510
T19S1	squamulose	blue	109	2906
T19S2	squamulose	blue	1060	4416
T19S3	squamulose	blue	531	7322
T19W1	foliose	green	12	
T19W1	squamulose	blue	365	377
T19W2	squamulose	blue	537	537
T19W3	foliose	green	2135	
T19W3	squamulose	blue	1225	3360
T25N1	foliose	green	4006	
T25N1	squamulose	blue	2510	6516
T25N2	foliose	green	2315	
T25N2	squamulose	blue	3861	6176
T25N3	foliose	green	1148	
T25N3	squamulose	blue	5258	6406
T25E1	squamulose	blue	174	174
T25E2	squamulose	blue	644	644
T25E3	fruticose	red	29	

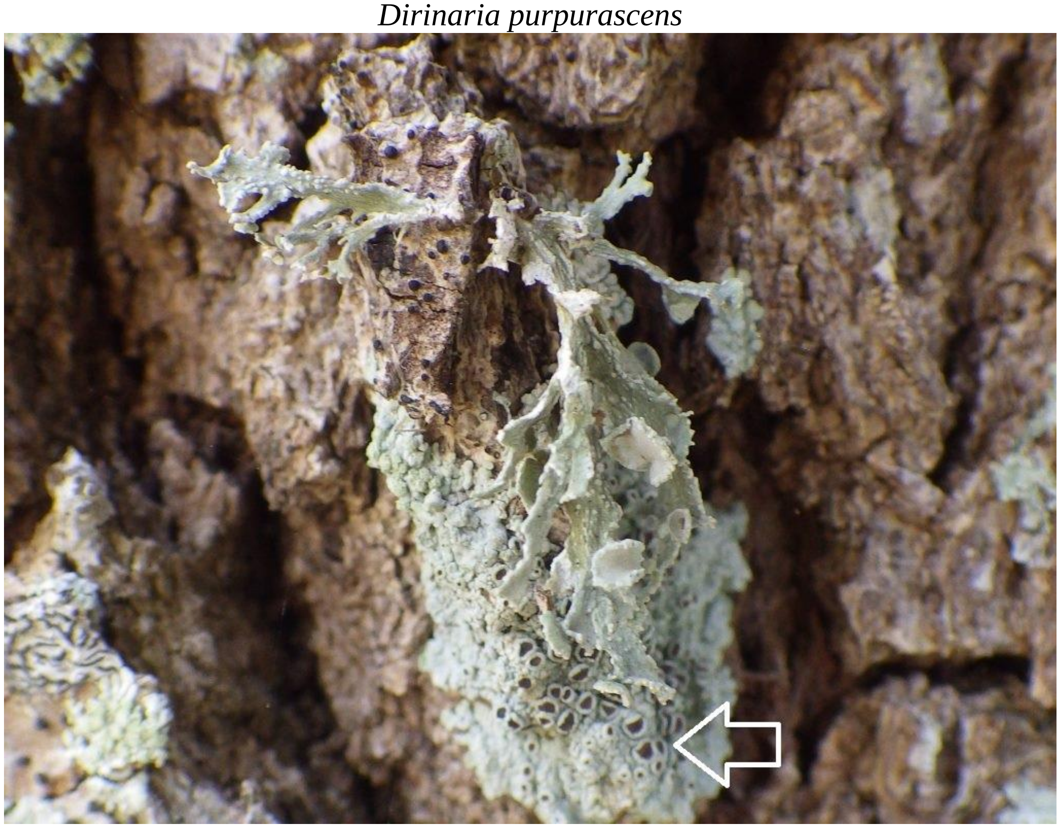
T25E3	foliose	green	151	
T25E3	squamulose	blue	1937	2117
T25S1	squamulose	blue	369	369
T25S2	foliose	green	1147	
T25S2	squamulose	blue	1604	2751
T25S3	foliose	green	129	
T25S3	squamulose	blue	2107	2236
T25W1	squamulose	blue	158	158
T25W2	foliose	green	1200	
T25W2	squamulose	blue	2310	3510
T25W3	foliose	green	2345	
T25W3	squamulose	blue	2990	5335
T29N1	squamulose	blue	67	67
T29N2	foliose	green	23	
T29N2	squamulose	blue	240	263
T29N3	foliose	green	1592	
T29N3	squamulose	blue	940	2532
T29E1	foliose	green	63	
T29E1	squamulose	blue	193	256
T29E2	squamulose	blue	773	773
T29E3	squamulose	blue	371	371
T29S1	foliose	green	40	
T29S1	squamulose	blue	158	198
T29S2	squamulose	blue	200	200
T29S3	squamulose	blue	271	271
T29W1	foliose	green	47	
T29W1	squamulose	blue	150	197
T29W2	squamulose	blue	144	144
T29W3	foliose	green	444	
T29W3	squamulose	blue	621	1065

Appendix III. Photographs of identified species

Dirinaria confusa

Dirinaria picta

Dirinaria purpurascens



Parmotrema cristiferum

Parmotrema perforatum

Parmotrema praesorediosum

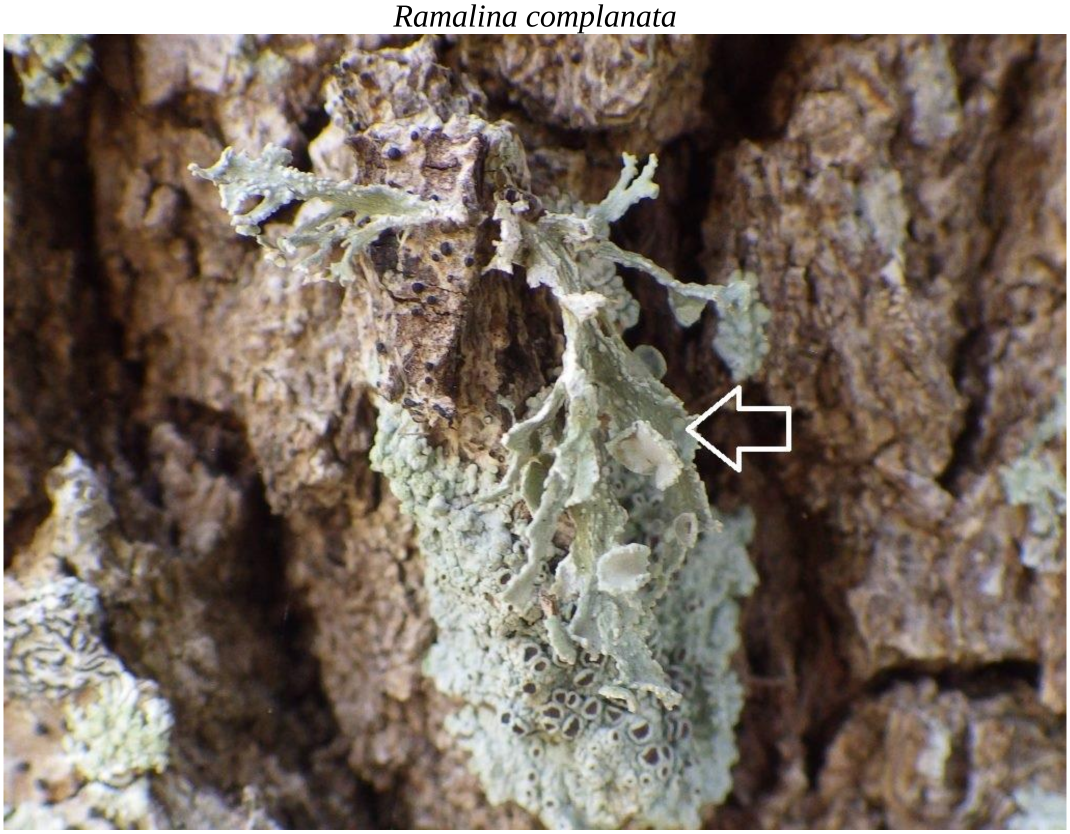
Parmotrema reticulatum

Parmotrema subrigidum

Parmotrema tinctorum

Ramalina cf. *americana*

Ramalina complanata



Ramalina denticulata

Ramalina peruviana

Ramalina stenospora