THERMAL PROPERTIES OF LEAF TRAITS

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ABSTRACT

Plants adapt to survive under a variety of environmental conditions by changing some of their traits. Also their emissivity influences how much energy they re-radiate to the environment. Subtle changes in leaf traits could affect the emissivity of a leave and through that their radiation. Some research has pointed out that thermal emissivity spectra can be associated with leaf surface structure traits. Therefore, leaf anatomical traits could correlate with thermal emissivity, but little is known regarding the relation between leaf traits and emissivity of thermal radiation. This study aims to estimate the correlation between emissivity at the thermal infrared part of the electromagnetic spectrum and leaf anatomical traits. Also it tried to detect groups of bands where a strong relationship between emissivity and leave traits can be found.

From eight herbaceous species (four tropical and four temperate species), leaf emissivity spectra were determined from Directional Hemispherical Reflectance (DHR) spectra, measured by a Bruker spectrometer using Kirchhoff's law ($\varepsilon=1-R$). Of every measured leaf, traits were afterwards quantified using a microscope. Stomatal density and size, vein diameter and cuticular membrane thickness are related to plant transpiration and therefore temperature regulation, so it was assumed this might influence emissivity spectra.

The correlation coefficient between each anatomical trait and emissivity was estimated at all measured bands (1.4 – 16 μm). The band with the largest absolute correlation coefficient within the TIR wavelength range was selected for further analysis. Traits showed a range of correlations with emissivity, but some traits had comparable correlations at their optimal bands: the R-value of vein horizontal diameter was -0.93, vein vertical diameter was -0.83, vein area was -0.90, stomata area was -0.93, stomata density was 0.76, top cuticular membrane thickness was -0.86, and bottom cuticular membrane thickness was -0.39. In general, the tropical plants had lower emissivity than temperate plants, but this difference was not significant, except in the region 9.59- 9.62μm and 9.65-10.25μm. In addition, I tested the correlation between every leaf trait. The results suggested strong correlations between leaf traits. For future studies, trichome density should be tested. Trichome density was not tested in this study given difficulty in determining this trait, but preliminary results suggested an effect in the 5-9 μm region. Also the data collected did not show a perfect spread of leaf traits, so more samples might be tested to fill those gaps in the range of traits to make the insights on correlations between emissivity and leaf traits more conclusive.

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1. INTRODUCTION

1.1. Background and significance

Leaf traits are properties of plants for supporting their survival, and these traits have been used in many studies performing research on botany, agriculture, climate change impacts and other ecological studies (Violle et al., 2007). These traits can change over time. They are affected by environmental factors and change in response to their dynamic surroundings, which has been evidenced by many studies (Diaz et al., 2004; Wenzel et al., 2012; Wright et al., 2004). The correlations between leaf traits and their changing surroundings (like climate), has increasingly interested plant ecologists and physiologists during the last three decades (Field, 1986; Grime et al., 1997; Hättenschwiler et al., 2011; Körner, 1995; Niinemets, 2001; Reich et al., 1992, 1997; Schulze et al., 1994; Wright & Westoby, 2002; Wright et al., 2005). However, the flexible nature of leaf traits makes the continuous monitoring of them difficult, especially when there is an interest to detect leaf-traits at a large scale or extent. Manual trait measurements under a microscope in the laboratory cannot satisfy such requirements. A main reason is that manual measurements are time consuming, and the method is destrictive, making continuous measurements of the same leaf impossible. Another drawback of laboratory measurements is the dependence on skilled operators which themselves induce variability impacting the accuracy of the results. Therefore, new methods of leaf traits measurement are required. This thesis analyses the correlation between emissivity and leaf traits which may help in developing a new measuring method.

1.1.1.1. Leaf anatomical structure

The term leaf traits covers many different types of traits. Two main types are functional traits and anatomical traits. Leaf anatomical traits directly reflect leaf microstructure, and trait values depend on indexes of microstructural features. Ribeiro da Luz and Crowley (2007) pointed out that emissivity spectra at Thermal Infrared (TIR) bands are associated with leaf surface structure aspects. Therefore, leaf anatomical traits could relate to thermal emissivity properties, but these relationships are unclear. This thesis measured emissivity and seven anatomical traits of leaves, and tested the correlations between them. In the following section, a brief introduction of leaf anatomical structure is presented, which relates to the seven analysed traits.

From a cross section, observed under a microscope, a leaf generally consists of four layers. From top to bottom the layers are named: upper epidermis, palisade mesophyll, spongy mesophyll and lower epidermis. Both upper and lower epidermises protect the leaf and are mostly covered by a waxy cuticle, which can efficiently prevent water lost. Stomata lie on the epidermal layer and are flanked by two guard cells. Guard cells regulate the flow of gas exchanging (including the amount of water) by controlling the size of the stomata opening. Some plant have trichomes (hairs) appending on the epidermis, which can reflect solar radiation, reduce evaporation, and in some moist locations trichomes can also collect water from the air. The veins of a leaf consist of the assemblage of vascular tissues laying in the sponge mesophyll layer. Transporting fluids is the main function of veins (Corson et al., 2009; Durand, 2006; McCulloh et al., 2003).

Three organs of leaf structure are involved in this analysis: cuticular membranes, stomata and veins. Seven specific traits are extracted to quantify the size of the above three organs: top cuticular membrane thickness, bottom cuticular membrane thickness, stomatal area, stomatal density, vein horizontal diameter, vein vertical diameter and vein area.

1.2. Leaf traits

The seven tested traits in the thesis were chosen for the following reasons. After initial microscope observations, we found that most leaf vein cross sections were not a circle but more ellipsoids. Therefore, we measured in two directions, horizontal and vertical diameter. Also we added "vein area", because there was a remarkable difference between tropical and temperate plants regarding this trait.

Becker et al. (1986) noted that some plant leaves have a thicker cuticular membrane on the top compared to the bottom. That is often the case in tropical species. On one hand, tropical plants have to resist water losses under high solar radiation, on the other hand these plants have to allow transpiration to keep their leaves cool. So tropical plants usually have a thicker cuticular membrane on the top to resist water loss, while carrying a thinner cuticular on the bottom for transpiration. Hence, I measured the thickness of the leaf cuticular membrane on two sides instead of a general thickness.

Finally, during the stomata density measurements, I found stomata area showed significant difference between tropical and temperate plants. Therefore, stomata area was included into the analysis. So, in total, seven traits were measured and their correlations with emissivity analysed in the thesis.

1.2.1. Emissivity and plant physiology

Laboratory measurements (Hecker et al., 2011) have shown that TIR emissivity spectra are useful for identifying many minerals, rocks, and other solid materials. Also plant leaves have been shown to vary in this part of the spectrum (Ullah et al., 2012a). However, the spatial variability of emissivity spectra of material surfaces is largely unknown (Schlerf et al., 2012) . Some research showed that analysis of leaf reflectance within the Near Infrared (NIR) region can be used to evaluate the effects of leaf structural properties on reflectance, as opposed to leaf chemical constituents such as chlorophyll and water (Curran et al., 1992; Hunt Jr & Rock, 1989; Purposes, 1970). Hallam and Chambers (1970) and Villena et al. (2000) illustrated that thermal infrared transmission spectra previously have been used to help understand the composition and the structure of leaf surfaces. Ribeiro da Luz and Crowley (2007) also pointed out the thermal emissivity associated with leaf surface structure aspects.

A plant captures energy from the sun, but only a part of this energy will be assimilated. The amount of absorbed radiation can be calculated using the absorption rate. This absorbed energy will be used in two major ways, one is to provide energy to satisfy plant maintenance respiration and net productivity, and the other one is heating the leaf. In case of excess heat, the plant has to dissipate the heat to reduce overheating and cool the leaves. Plants dissipate heat mainly via three ways: long-wave radiation (thermal emissivity), heat convection into the air and transpiration (Johnson, 2012). Transpiration is the dominant way, and relates to a series of organs cooperating, such as the vein architecture and stomatal density. To be specific, plants pump water and nutrients from the soil by roots, through the xylem in the stem and finally transport it to the leaf cells through the veins. Some of the water in leaf cell is using for plant photosynthesis, while the majority of the water evaporates and escapes through by stomata. That process

provides a relatively stable temperature condition in the leaf to maintain photosynthesis to survive under varying environments (Watson, 1933; Yang et al., 2012).

1.3. Research problem statement

There are many unknowns related to TIR spectroscopy and plant traits, which need clarification to progress our knowledge on the usability of TIR spectroscopy for vegetation monitoring. For example, which leaf anatomical traits correlate with emissivity, and are they positive or negative? Which trait has a stronger influence on emissivity and at TIR band or group of bands?

As I mentioned in the previous section, transpiration is a vital way for plant to lose excess heat. That only works under conditions with sufficient water. When plants live in a cold environment, they will aim to conserve energy (heat). However, plants cannot avoid transpiration completely, because they have to photosynthesize to satisfy respiratory needs, a process that also induces transpiration. Some plants living in temperate areas, for instance, adapt themselves to cold conditions by coating their leaves with trichomes and reduce emissivity to prevent heat loss. On the other hand, tropical plants living in warm conditions can capture a lot solar radiation, so they do not need to conserve energy but have to dissipate excess heat.

In this experiment, I analysed plants living in the same warm conditions with sufficient water, and measured their emissivity and traits. Because temperate plants originally live under cooler conditions with less water compared with tropical plants, they may invest less in transpiration infrastructure, such as small stomata and narrow vein diameters. On the other hand, tropical plants probably carry large stomata with large vein diameter. Therefore, we do not know what the result will be when tropical and temperate plants growing under similar conditions will be compared. That may lead to hypothesized trends as described in the "Research question and hypotheses" section.

1.4. Research objective

The objective of this study is to estimate the correlations between leaf anatomical traits and spectral emissivity using hyperspectral bands in the TIR. Also, the research aims to detect groups of bands that show the highest correlation with those traits. Furthermore, based on these bands, the research aims to test linear regressions between emissivity and traits.

Seven leaf anatomical traits (vein horizontal length, vein vertical length, vein area, stomata area, stomata density, top and bottom cuticular membrane thickness) are analyzed and six following specific questions are posited. This thesis analysed herbaceous plants from tropical and temperate area, so the results might not be compareable with plants from deserts or other dry areas.

1.5. Research questions and hypotheses

There are six specific research questions that need to be tested. The first five questions are about the relationships between emissivity and leaf anatomical traits, and the last one is the comparison of the emissivity between tropical and temperate plants. For all the questions a possible hypothesis is stated.

Question one: Is there a correlation between emissivity and vein diameter?
Question two: Is there a correlation between emissivity and vein area?

Question three: Is there a correlation between emissivity and single stomata area?
Question four: Is there a correlation between emissivity and stomata density?
Question five: Is there a correlation between emissivity and thickness of cuticular membrane?
Question six: Do temperate plants have a higher emissivity than tropical plants?

The first two questions focus on vein traits. Veins deliver water and nutrients to leaf cell, so its size impacts the water content of the leaf. Therefore, larger veins provide leaves with more water for plant transpiration. From the previous work (Ullah et al., 2012b), leaf emissivity increased with increasing water content of leaf. Therefore, our hypotheses is that there are positive correlations between emissivity and leaf vein traits (including diameter and area). However, from the view of plant transpiration, opposite results might be expected. Plant transpiration is a process of evaporating water that cools a leaf. More water content of a leaf will stimulate the plant to transpire water and so leaf temperature will decrease. A risk of a low temperature might therefore be accompanied by a low emissivity to reduce further heat loss. In other words, with high transpiration, emissivity of the leaf will drop. Hence, an alternative hypotheses could be that a negative correlation exists between emissivity and leaf vein traits.

The same alternative hypotheses can be suggested for other leaf traits. For stomata traits (questions three and four), Xu and Zhou (2008) suggested a negative correlation with leaf water. So stomata density may be negatively correlated with emissivity. But low stomata density also relates to less transpiration, which will lead to an increased temperature of leaf. From this point, stomata density might stimulate emissivity to lose excess heat. Stomata density and stomata area are probably negatively correlated with each other, because a negative correlation exists between stomata density and stomata diameter as proven by Galmés et al. (2007). Therefore, if stomata density shows a negative correlation to emissivity, then stomata area will show a positive correlation. In the case of the opposite situation, stomata density is positively correlated with emissivity, while stomata area may have a negative correlation with emissivity.

Cuticular membrane thickness (question five), can protect plants from extreme conditions, a thicker cuticle helps to preserve the loss of water from a leaf and to resist intensive solar radiation. Therefore, in a warm environment, the preservation of water loss decreases the plant transpiration process, the temperature of leaf will be higher and the emissivity might increase to lose excess heat. In that case the thickness of cuticular membrane is positively correlated with emissivity; but considering cuticle can decrease the absorption of radiation and therefore reduce the buildup of heat in the leaves, a thicker cuticle may be also negatively correlated with leaf emissivity.

It is hard to predict which correlation is correct, as both sides have a reasonable explanation. The only way to determine the reality is by measuring and testing in the laboratory.

For the last question (question six), tropical plants show a more intensive transpiration than temperate plants (Downes, 1969; Schreiber & Riederer, 1996), more water will be evaporated from the leaf surface, therefore, tropical plants probably contain less water in the leaf. Based on that reason, there is only one hypothesis for this question: the emissivity of tropical plant should be lower than that of temperate plant.

1.6. Flowchart

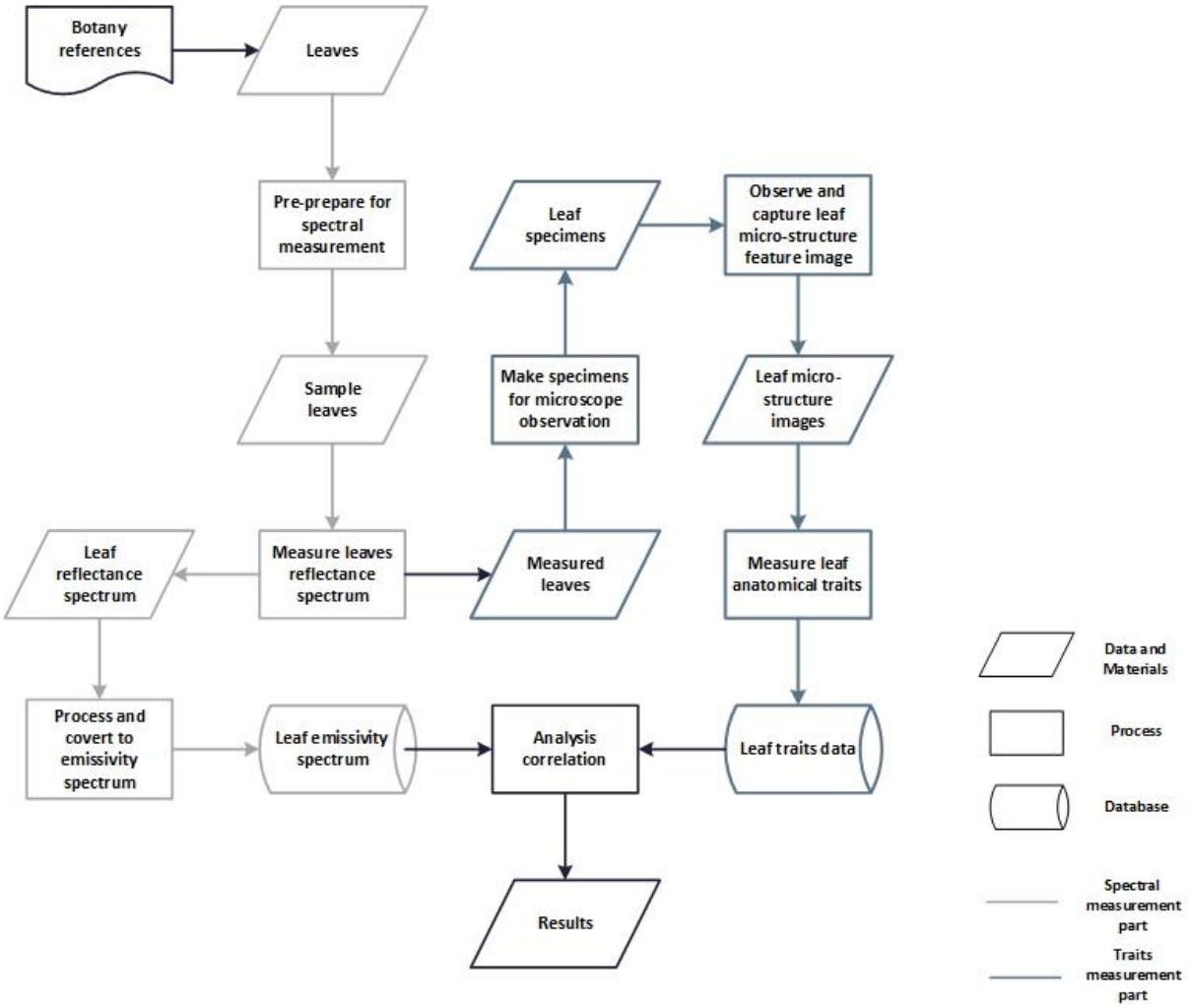

Figure 1-1: Flowchart

2. MATERIALS AND METHODS

In this thesis, leaf spectral measurement and leaf anatomic trait measurements were made. These measured results were used for statistical analysis.

2.1. Study sites and leaf sampling

The experiment was located in Enschede, the Netherlands, and took place between September 23 and October 11, 2013. Eight herbaceous species, four tropical and four temperate species, were used in the experiment.

2.1.1. Species selection

At the beginning of the experiment, determining which species should be selected and how many leaves had to be measured per species were an essential issue, because of time limitation and specific reference information lacking (like some detailed recordings of specific leaf trait was missing). Therefore, we started with a random selection of species from the ITC building and garden. The whole species selection consisted of three stages. At stage one we applied a random selection of species. Around Enschede, a temperate oceanic climate prevails, so most outdoor species were temperate plants. And indoor evergreen plant like in any other areas were tropical. Four species were selected to continue the further measurement. At stage two (gap covering selection), two more temperate species were selected from the ITC garden, according to experimental results of previous measurement. These two species filled up a few gaps in leaf anatomical traits values. At stage three (balance sample selection) because of the unbalances selection (two tropical and four temperate species), two more tropical species were added to this experiment so that a comparison between tropical and temperate species would be made possible. Three tropical species, 6 pot plants per species (18 pots in total) were brought from a plant market. After testing anatomical traits, two of them were selected.

Finally 80 leaves were measured, divided over 8 species (Table 2-1 and Figure 2-1) with original locations distributed from tropical to temperate areas, which lead to a significant variation of leaf anatomical traits.

Table 2-1: Eight plant Species, their ordering number1, code, scientific name and original location

Species Number	Species Code	Species Scientific Name	Origin Location
1	Sp1	Stachys Byzantira	Temperate
2	Sp2	Dieffenbachia	Tropical
3	Sp3	Gezanium Macrorrhizum	Temperate
4	Sp4	Aglaonema Mary Ann	Tropical
5	Sp5	Geranium Himalayense 'Gravetye'	Temperate
6	Sp6	Persicazia Amplexicaulis 'Firetail'	Temperate
7	Sp7	Fittonia Verschaffeltii	Tropical
8	Sp8	Calathea Rufibarba 'wavestar'	Tropical

Figure 2-1: Eight species photos, Species1, 2, 3, 4 and Species5, 6, 7, 8 (from left to right)

2.1.2. Leaf selection

As the leaves vary in shape, size and health conditions, these criteria were considered for the leaf selection: firstly, choosing healthy and clean leaves. As any slight difference might lead to variations on emissivity spectra, such as, a wet healthy leaf might show lower emissivity at MIR bands compared with a dry leaf, and dust particle might decrease leaf emissivity. Secondly, choosing similar size leaves with width exceed 30mm as possible for every species. Since the sample port of Bruker spectrometer was 30mm in diameter (with a 25mm in diameter measuring spot), the leaf width should cover over the whole area of measuring spot. Besides, similar size of leaves is related to a similar generation, which would help to minimize variations on one species. Thirdly, if one piece of leaf was too small to cover over the measuring port, two overlapped leaves were used instead.

2.2. Leaf spectrum measurement

2.2.1. Equipment and software

The Bruker VERTEX 70 FTIR (Fourier Transform Infrared) spectrometer (Figure 2-2) was used for leaf DHR (Directional Hemispherical Reflectance) spectrum measurements. The software of OPUS, HyPpy and ENVI 5.0 were used for spectrum data processing.

Figure 2-2: Bruker VERTEX 70 FTIR spectrometer

The Bruker spectrometer can be equipped with optical components to cover the spectral range from 1.4 to 16.0μm with 6612 spectral bands. This spectrometer system continuously flows Nitrogen gas to purge the impact of water vapor and carbon dioxide. In this case, a Globar (150W) was used as an external source to provide infrared light. Infrared light was emitted from the Globar and directed into an interferometer for separating incident light beams. We used a KBr Beamsplitter with Ge coating as the interferometer, which could modulate infrared light into a wide spectral region (from 0.2μm to 20μm) with different angles of light incidence. An integrating sphere with gold coating was set as a connector between the spectrometer and external port, which sphere coated with gold for providing a highly diffuse reflectance surface (Hecker et al., 2011; Ullah et al., 2012a). After the interferometer split, infrared light passed through the integrating sphere and was focused onto the sample at the external port under the integrating sphere. Then the light reflected from the sample to the Mercury Cadmium Telluride (MCT) TIR detector (Figure 2-3). The MCT detector (cooled with liquid Nitrogen) was used for measuring leaf DHR spectrum, in the wavelength region from 1.4 to 16.0μm.

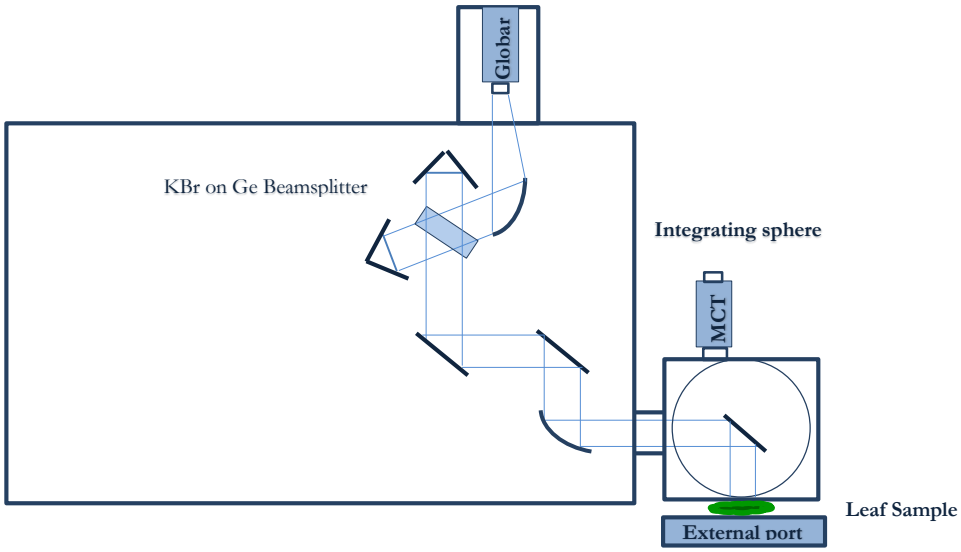

Figure 2-3: Bruker VERTEX 70 FTIR spectrometer layout and beam

The software of OPUS monitored the whole process of leaf HDR measurement and recorded all raw reflectance spectra of leaf samples. Also, all spectral measuring parameters and path directions were set by OPUS. Raw spectra were repeatedly measured for a higher accuracy, and an averaged spectrum of each leaf was calculated as the final spectral result. For that purpose, HyPpy software was used. In addition, HyPpy converted the format of spectral data to ScRf (Simple Channel Reference) and ScSm (Simple Channel Simple) files for offsetting daily error of spectroscope process. That error offsetting process was achieved by Python in ENVI5.0 and the emissivity spectral library was built up by the Spectral function in ENVI5.0.

2.2.2. Spectral Measurement Preparation

2.2.2.1. Instrument preparation

To ensure spectrometer system keep operating stably, the external source (Globar) and the instrument were turned on more than one hour in advance of the first measurement. During the whole measuring process, the MCT detector was cooled with liquid nitrogen and the purged Nitrogen (N_2) gas flowed away interfering particles on the surface of the object. N_2 Gas was turned up to 150 L/h at the beginning of system operating, then from ten minutes before the first measurement, N_2 gas flow was decreased to 100 L/h (this flow value was kept constant during spectral measurement).

2.2.2.2. Sample preparation

The temperate samples would be kept indoor at least 30 minutes before DHR spectrum measurement, for increasing the leaf temperature close to room temperature. This step was necessary because temperate plants lived outdoor, and the temperature outside was around 15 Celsius degree lower than room (Figure 2-4). In order to keep the quantity of water content and the state of health, all the temperate leaves were kept in airtight plastics bags during warming up time. It was tested whether this conservation measure would affect the leaf water content by performing an experiment with a few leaves. For these leaves, their weight mostly stayed in a constant level. Therefore, the leaf water content was assumed keeping stable with covered by an airtight plastics bag.

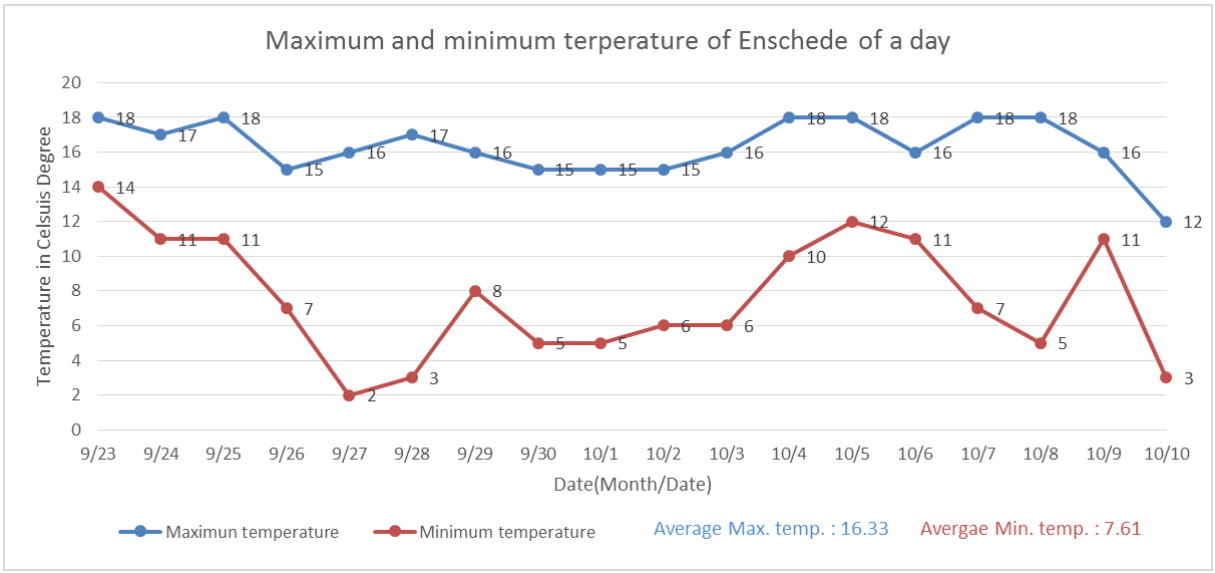

Figure 2-4: Maximum and minimum temperature of Enschede in one day from 23rd September to 10th October of 2013

2.2.3. DHR spectrum measurement

All the measurement started with standard calibration measurement using the infragold for reference, and two minutes were needed to purge with Nitrogen gas before every measurement. Following that standard calibration, leaves replaced the infragold under a sampling spot for spectral measurement. For both infragold standard calibration and sample measurement, measured object were as close as possible to the sampling spot for avoiding the background interference.

The spectra would be recorded from wavelength 1.4 to 16.0μm. But spectral signals were noisy at both the head and the tail of wavelengths. Therefore, the spectral range used for analysis was from 2.5 to 16.0μm. In this way, the standard setup of the spectra was measured in useful range (4,000cm−1 to 625cm−1) with a 4cm−1 spectral resolution. According to previous experimental experience, 512 scans could satisfy the infragold reducing errors from system with highly-reflectivity and samples with less reflectivity. Four repetitions (4*512 scans) could provide a sufficiently high signal-to-noise ratio (SNR) (Hecker et al., 2011).

Open port measurement would leave nothing under the sampling port to measure indoor environmental interference, which was generally regarded as daily measurement used for offsetting systematical errors or bias.

2.3. Leaf anatomical traits measurement

All trait measurement was estimated by the capturing leaf microstructure images and computing the image size.

2.3.1. Equipment and software

The Leitz SM-LUX Microscope and Sony DXC-151P CCD color video camera (Figure 2-5) were used for leaf anatomical trait observation and recording. CamRecorder and ToupView software were used for recording and leaf anatomical traits calculation.

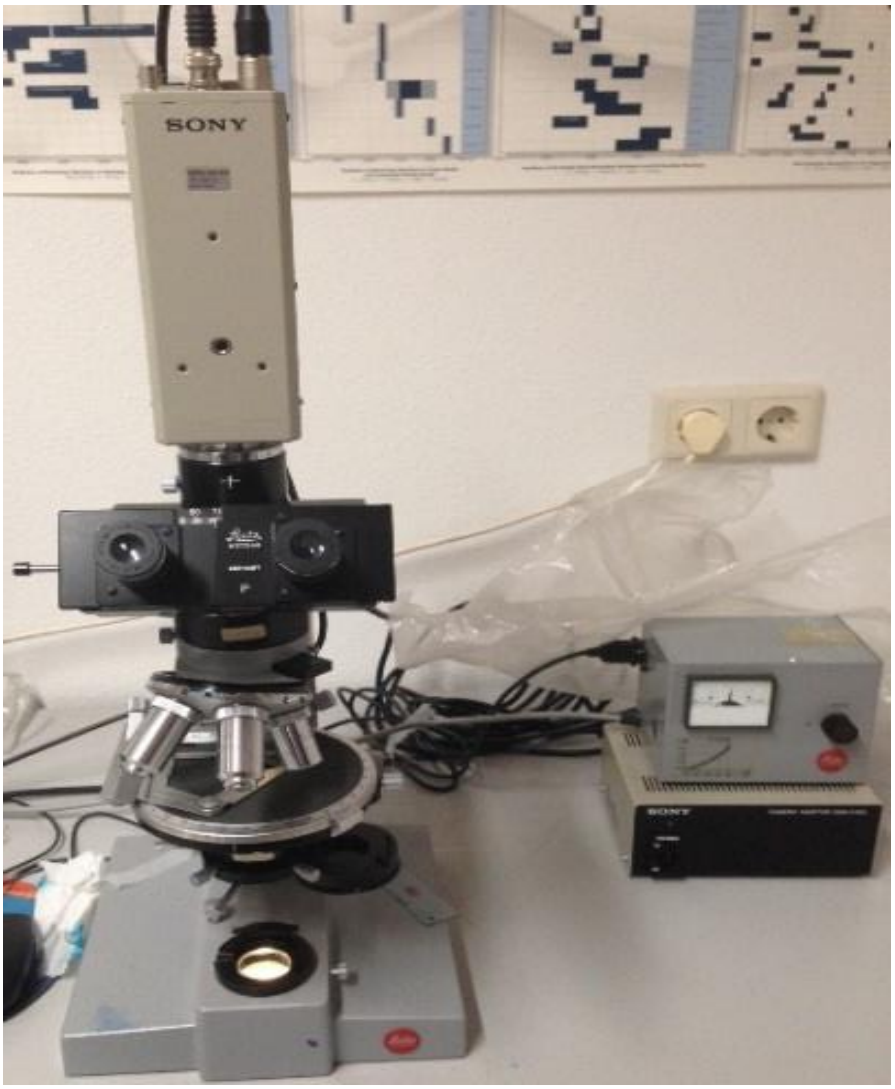

Figure 2-5: The Leitz SM-LUX Microscope and Sony DXC-151P CCD color video camera outlook

The Leitz microscope carrying 5 nosepieces provided 2.5, 4, 10, 25, 63 times amplification factors at different observation scale. A Sony camera transformed the way of observation, from eyespecies view to screen captured photo.

All leaf anatomical micro-photos and videos were captured and recorded by CamRecorder. The ToupView software could measure the thickness of leaf structure and the size of leaf organs at the micro-scale.

2.3.2. Specimens making

After spectral measurement, all the leaf samples (the same leaves that were measured spectra) would gave two kinds of specimens for microscope observation. One sort was vertical specimen, which specimen was sliced in vertical direction to observe vein and cuticular membrane characteristics (Figure 2-6). And the other sort was horizontal specimen, which was tore a portion of tissue off the leaf bottom skin to observe stomata features (Figure 2-6).

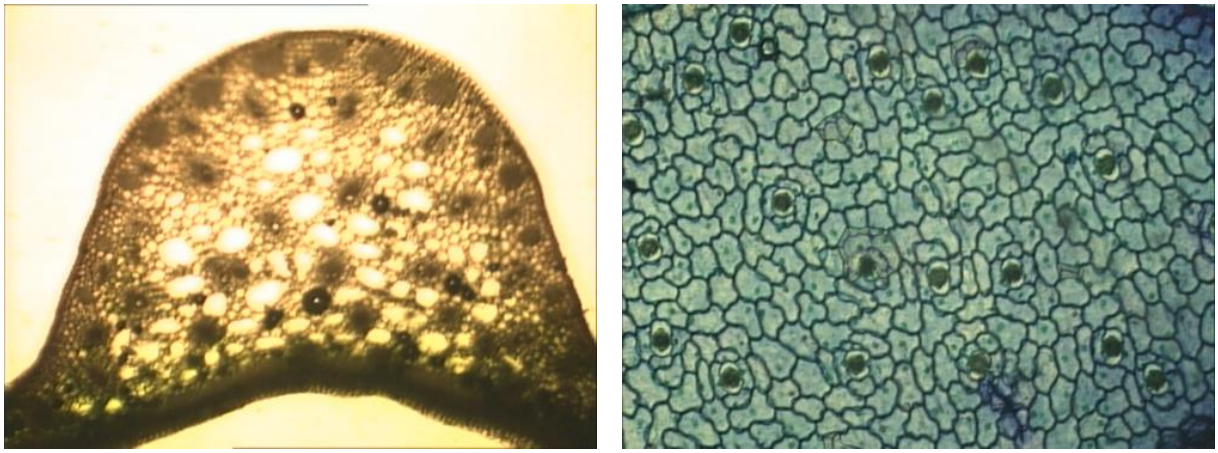

Figure 2-6: Vertical specimen with 40 amplification (left) and Horizontal specimen with 250 amplification (right)

2.3.3. Leaf anatomical traits measurement

Different leaf anatomical traits needed be measured with different amplifications. Also sometimes amplification was changed to adapt to different species for the same trait measurement. To consider about the accuracy, all the traits were firstly measured as pixel number, then transformed to the actual length or area unit by using a micrometer scale (100μm in 2μm divisions, TED PELLA, INC) based on their measured amplifications (Table 2-2).

Table 2-2: Pixel number and real length transform at different amplification scales

Amplification Factor	Pixel Number per 100μm	One Pixel Length (μm)	Area of Image View (mm^2) (Image: 480*640 Pixels)
25x	12.5	8.00	19.66
40x	20.0	5.00	7.68
100x	50.0	2.00	1.23
250x	125.0	0.80	0.20
630x	315.0	0.32	0.031

2.3.3.1. Vein horizontal length and vein vertical length measurement

The vein transection illustrated irregular geometries, but most transactions were close to an ellipse. Therefore, vein horizontal length and vertical length were measured and analysed in this study (Figure 2-7).

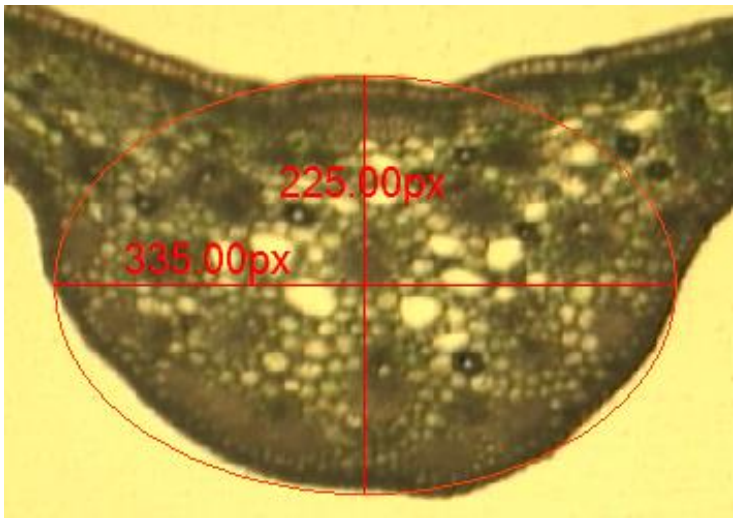

Figure 2-7: Vein horizontal length and vein vertical length measurement

2.3.3.2. Vein area measurement

The vein area could not be calculated by vein horizontal and vertical length directly most of time. That was because many leaf veins were composed of some vascular bundles that separated from each other. If we calculated vein area by multiplying horizontal and vertical length, the non-vein space area between the bundles would be included, which would result in overestimating the diameter of the vein. Therefore, we calculated each vascular bundle area independently and accumulated these areas together as the vein area (Figure 2-8).

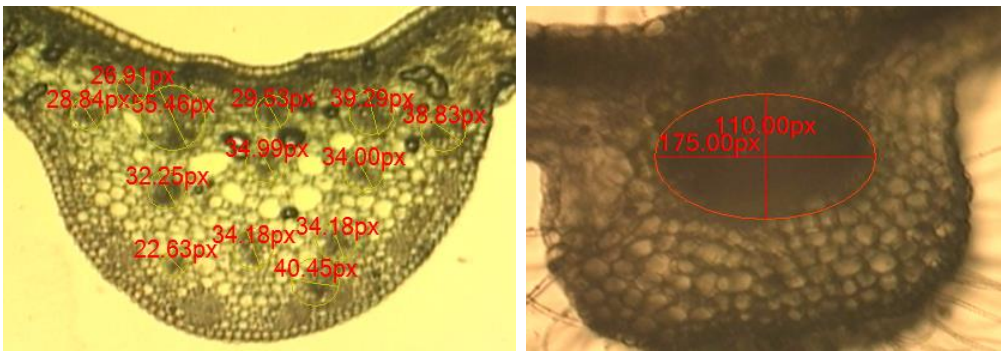

Figure 2-8: Vein area measurement

2.3.3.1. Cuticular membrane thickness measurement

The thickness of cuticular membrane varied on the top leaf side and the bottom leaf side for many plant species. Hence, in this thesis, the thickness of cuticular membrane was measured on both top and bottom sides of one leaf (Figure 2-9).

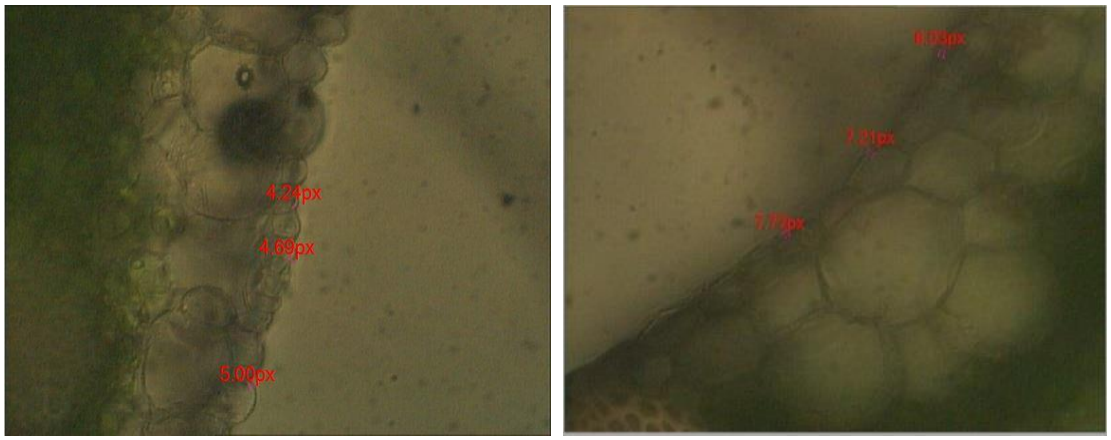

Figure 2-9: Cuticular membrane thickness measurement (left, bottom side; right, top side)

2.3.3.2. Stomata size and stomata density measurement

The size and density of stomata are various on different plant species, which means that they are dynamic traits and sensitive to the environmental changing condition of surroundings. But the adaptation of stomata size or density to the environment is not a rapid response (it needs several days or months). Therefore, in this case the two traits were considered as stable during the whole measuring process. Some species were hairy on the bottom side of the leaf, the long hair might lead to vertical tissue specimens not flat. That situation would reduce the accuracy of stomata traits calculation, because the microscope is used only for observing the flat specimen. Here, the video of stomata observation with focus changing was recorded, which helped to clearly show every stomata at different distance to the nosepieces. Hence, it was easy to count the number of stomata within the field of view, or to measure the size of stomata (Figure 2-10).

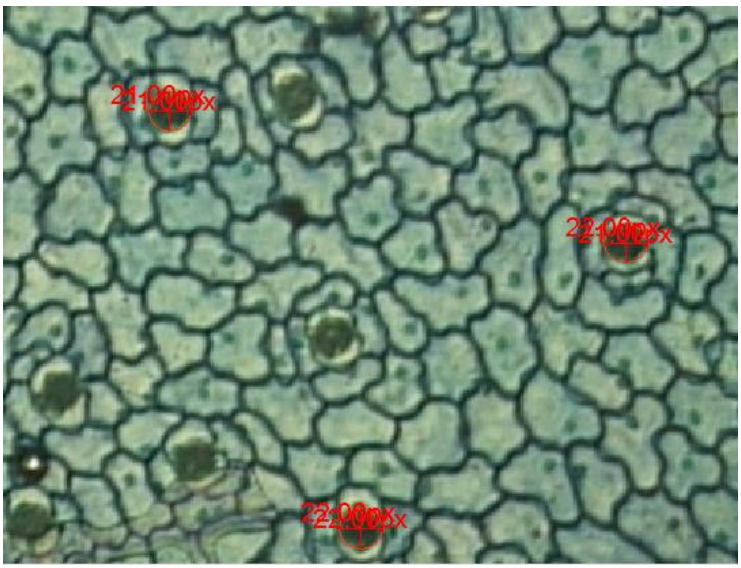

Figure 2-10: Stomata size and stomata density measurement

2.4. Statistical analysis

The database included in two sub-bases, leaf emissivity spectra sub-base and leaf anatomical traits sub-base. Each sub-base was collected from 80 samples.

2.4.1. Correlation analysis

In this thesis I calculated the correlation coefficient and coefficient of determination for testing the linear correlation of objects. A linear regression was used for modelling the relationship of emissivity and leaf traits. This correlation analysis test was applied for two aspects. One was to test between leaf emissivity and each anatomical trait, and the other was to test between each two different leaf anatomical traits. Also, in order to observe how well the regression equation represented the data, the coefficient of determination was required for both of aspects.

2.4.2. Difference analysis

Two-sample T-test with unequal variance was used for population difference analysis. That test was applied for testing if there was significant difference between tropical and temperate plant emissivity spectra.

3. RESULTS

3.1. All species leaf anatomical traits

Variation in the seven measured anatomical traits varied between species (Figure 3-1). All blue points were separated to eight groups by various species, and each point represented for one trait value of one sample leaf. And the 'H' and the 'V' of vein diameter represented for the horizontal and vertical length of vein. According to Figure 3-1, those sample points covered most part of trait range, though there were still gaps around 800-1200μm² and 2200-2600μm² where existed in stomata area, for instance.

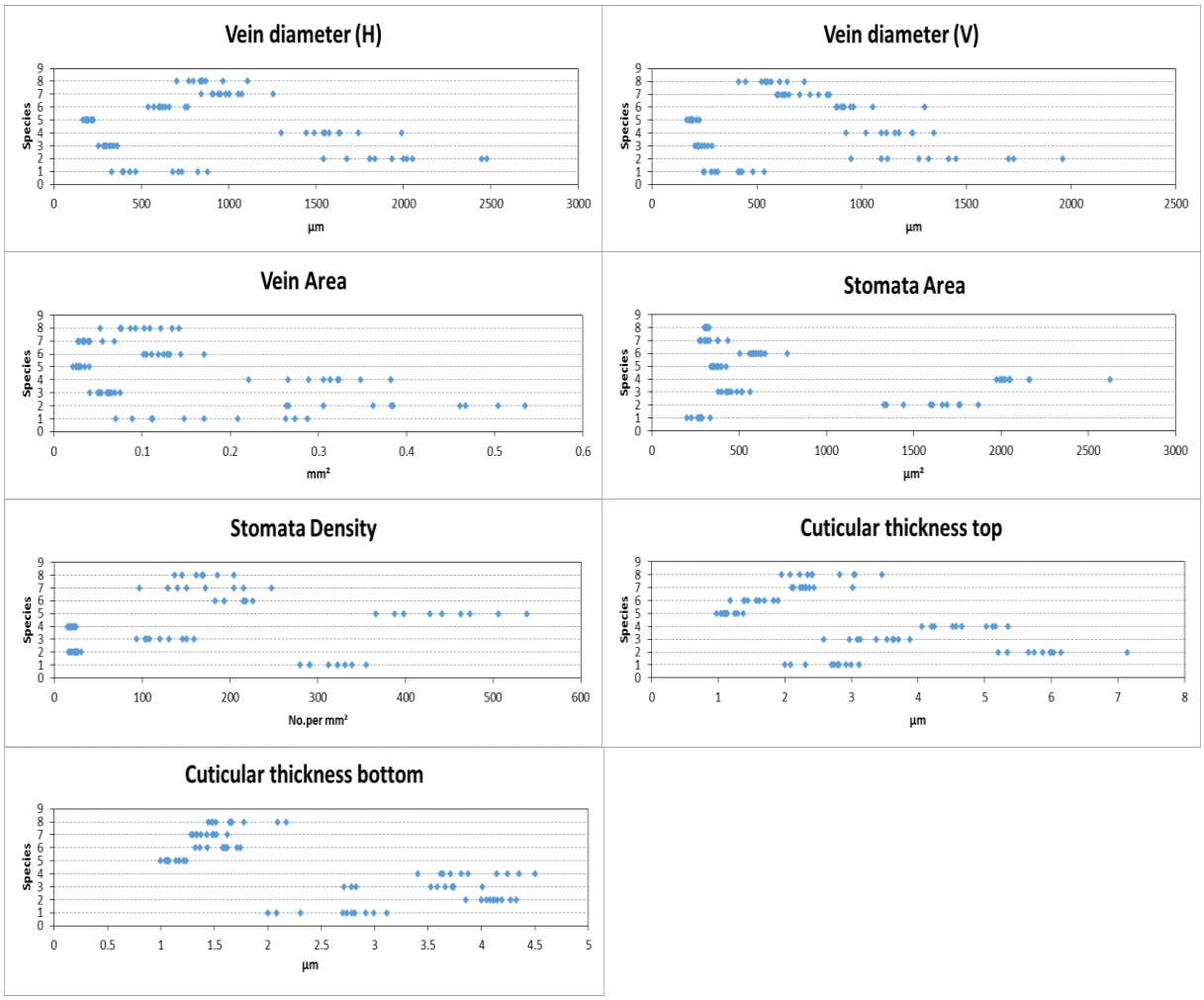

Figure 3-1: All leaf sample values distribution in each and anatomical trait

The average values of leaf anatomical traits per species varied between species (Table 3-1). Species 2 and 4 showed some distinct characteristics of trait compared with other species. For instance, these two species had larger veins and larger stomata with less stomata density than others. And the thickness value of top cuticular membrane within species 2, 4, 7, 8 was higher than the bottom side. Table3-1 also illustrates that species with a larger area of stomata had a lower density of stomata.

The tropical and temperate leaf anatomical traits values were averaged from species 2, 4, 7, 8, and from species 1, 3, 5, 6, respectively (Table 3-2). Table 3-2 illustrates significant differences between tropical and temperate plant in all seven traits. For example, tropical plants had three times vein diameters, three times

stomata area and two times cuticular membrane thickness compared to temperate plants. On the other hand, stomata density of plant living in temperate area was 13 times as intensive as tropical plant. In addition, temperate plants had similar thickness of top and bottom cuticular membrane, while cuticular membrane of tropical plants on the top side was thicker than the bottom side.

Table 3-3 shows the stomata area and stomata density values of all species and the averaged values from tropical and temperate plant. Table 3-1 suggests that a plant with a high stomata density has small stomata, I checked if the total stomata area in the field of view multiplied by the stomata density gave similar values for both tropical and temperate plants. The results are shown in Table 3-3. From the results, it clearly showed that total stomata areas (multiplied values) of Species2, Species4, Species7 and Species8 were smaller than other four species. For instance, the largest two stomata species, Species2 and Species4, had the smallest density and smallest multiplied values. On the other hand, the species with smaller stomata showed larger values in total stomata area. If we focused on comparison between tropical and temperate, tropical plant had larger stomata with less density than temperate plant, and had twice total stomata area as less as temperate plant.

Table 3-1: Average leaf anatomical traits of every species

Species No.	Vein Horizontal Length(μm)	Vein Vertical Length(μm)	Vein Area (mm^2)	Stomata Area (μm^2)	Stomata Density (No./mm^2)	Top Membrane Thickness (μm)	Bottom Membrane Thickness (μm)
1	584.40	366.70	0.17	269.53	313.58	2.65	2.65
2	1978.00	1401.50	0.39	1616.00	23.56	5.91	4.11
3	313.00	234.80	0.06	461.28	127.67	3.35	3.43
4	1589.50	1157.00	0.31	2106.62	19.94	4.69	3.93
5	193.46	188.18	0.03	375.10	444.39	1.14	1.12
6	635.00	972.20	0.13	609.98	209.14	1.58	1.55
7	967.40	708.20	0.04	319.64	163.65	2.35	1.42
8	859.20	557.20	0.10	311.67	164.84	2.58	1.69

Table 3-2: Average leaf anatomical traits of tropical and temperate plant

Plant	Vein Horizontal Length(μm)	Vein Vertical Length(μm)	Vein Area (mm^2)	Stomata Area (μm^2)	Stomata Density (No./mm^2)	Top Membrane Thickness (μm)	Bottom Membrane Thickness (μm)
Temperate	431.47	440.47	0.10	428.97	273.69	2.18	2.19
Tropical	1775.89	1265.32	0.34	1848.81	21.85	5.27	4.02

Table 3-3: Stomata area and density of species

Species Code	Species1	Species2	Species3	Species4	Species5	Species6	Species7	Species8	Tropical	Temperate
Stomata Area	269.53	1616.90	461.28	2106.62	375.10	609.98	319.64	311.67	1088.71	428.97
Stomata Density	313.58	23.56	127.67	19.94	444.39	209.14	163.65	164.84	93.00	273.69
Area*Density	84519.83	38098.90	58888.82	42002.08	166690.48	127571.11	52310.06	51373.95	45946.25	109417.56

3.2. Leaf emissivity spectrum and P-value graph

The emissivity spectra of eight species, and of tropical and temperate plants were averaged from each leaf for all 6612 bands, with wavelength ranging from 1.4μm to 16μm (Figure 3-2and Figure 3-4). According to Figure 3-2, species1 emissivity spectrum showed significantly lower than other seven species at Mid-Infrared wavelength range from 2.5 to 6μm. And more details of emissivity spectrum at TIR wavelength

range (8.0μm-14.0μm) were amplified as Figure 3-3 and Figure 3-5. The emissivity of tropical plants was lower than temperate plant at each TIR bands.

P-value of Two-sample T-test with unequal variance between tropical and temperate plant emissivity was showed in Figure 3-6. There were only two wavelength ranges (9.59- 9.62μm and of 9.65-10.25μm) at TIR bands, where P-value is less than 0.05 (Figure 3-7).

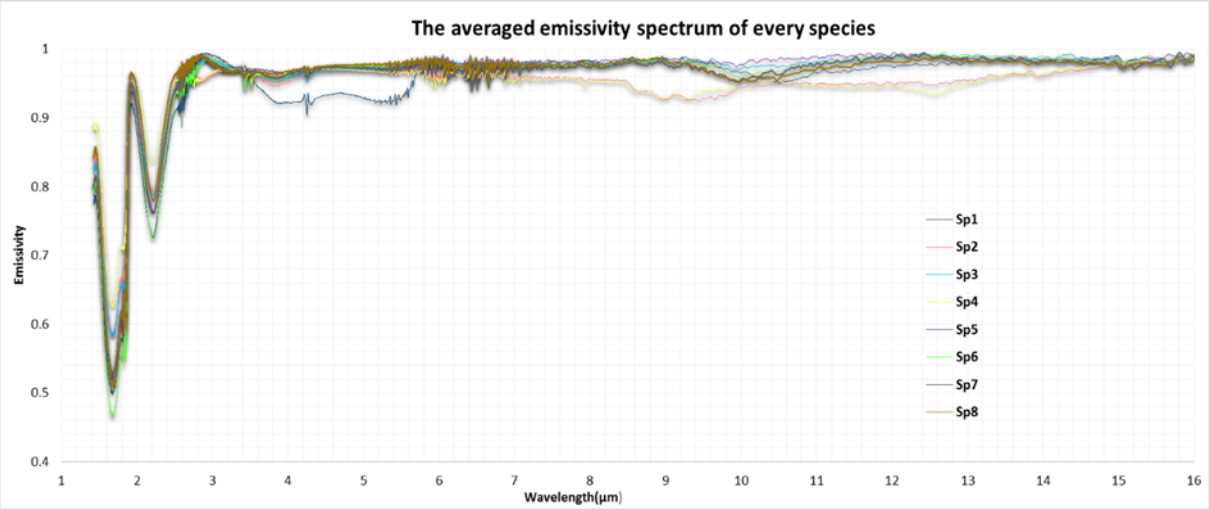

Figure 3-2: Averaged emissivity spectrum of eight species at 6616 bands

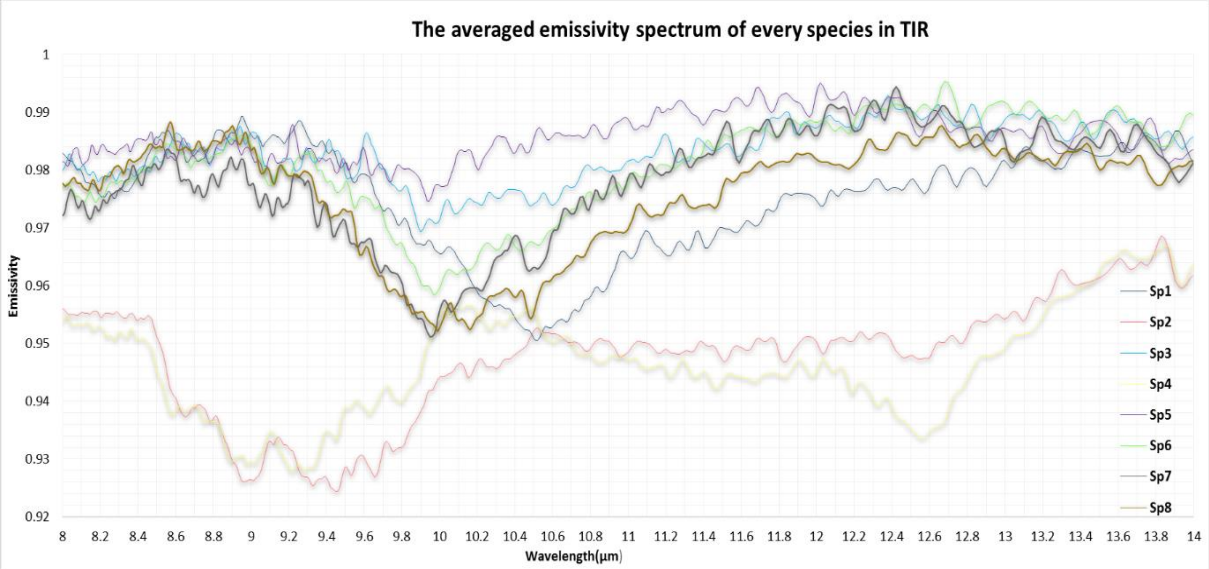

Figure 3-3: Averaged emissivity spectrum of eight species at TIR bands

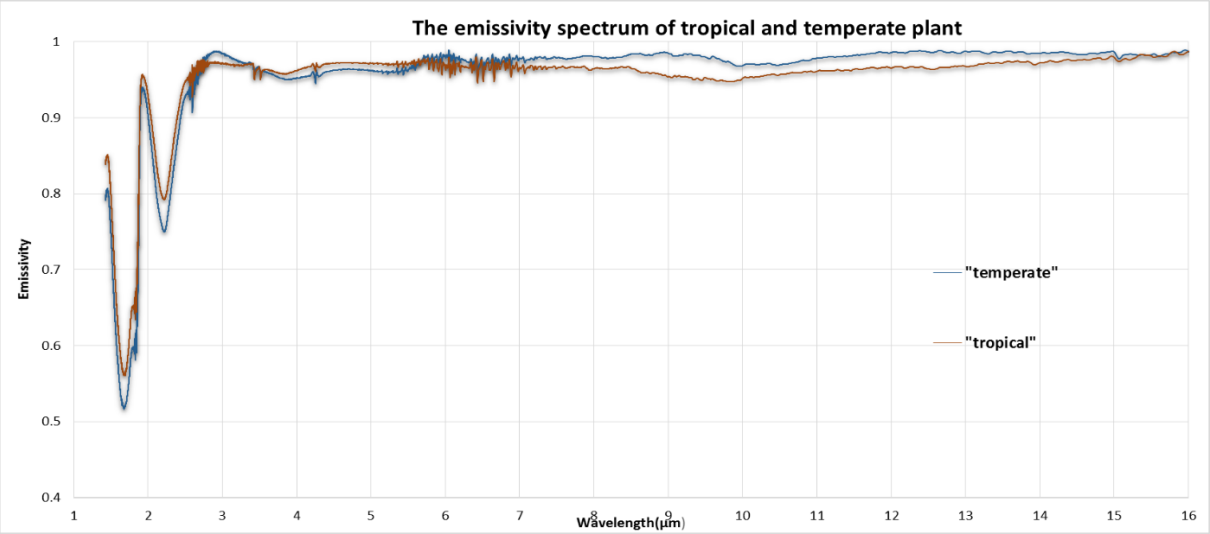

Figure 3-4: Tropical and temperate plant emissivity spectrum at 6612 bands

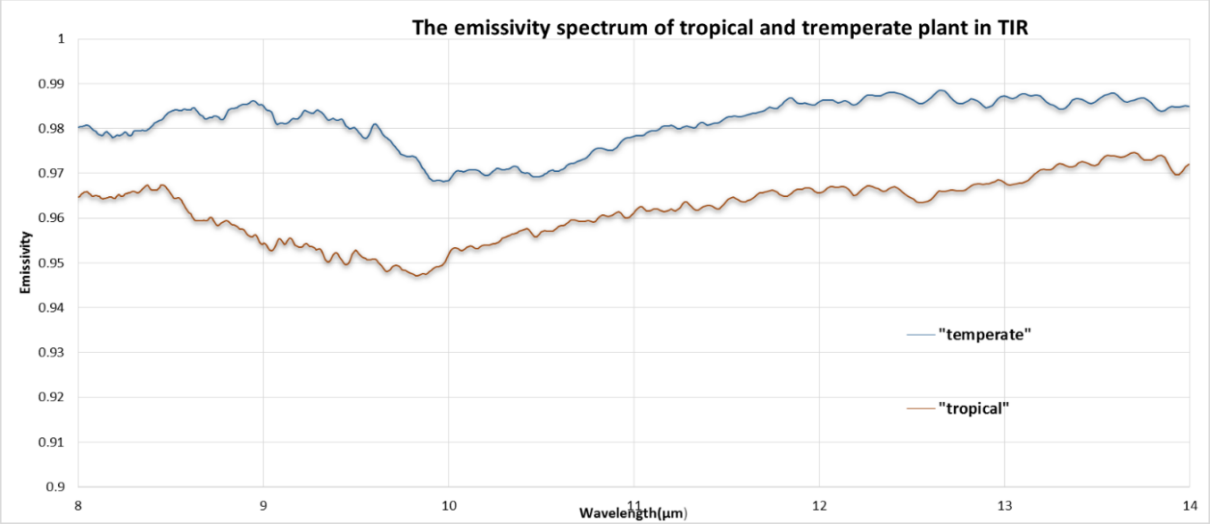

Figure 3-5: Tropical and temperate plant emissivity spectrum at TIR bands

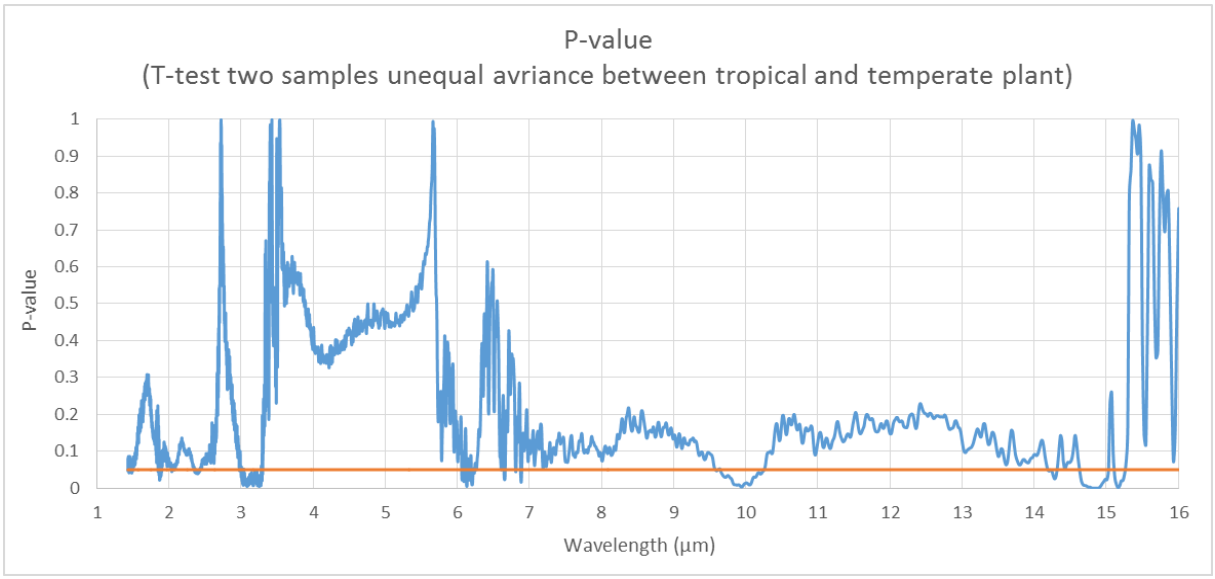

Figure 3-6: P-value of T-test between tropical and temperate plant emissivity

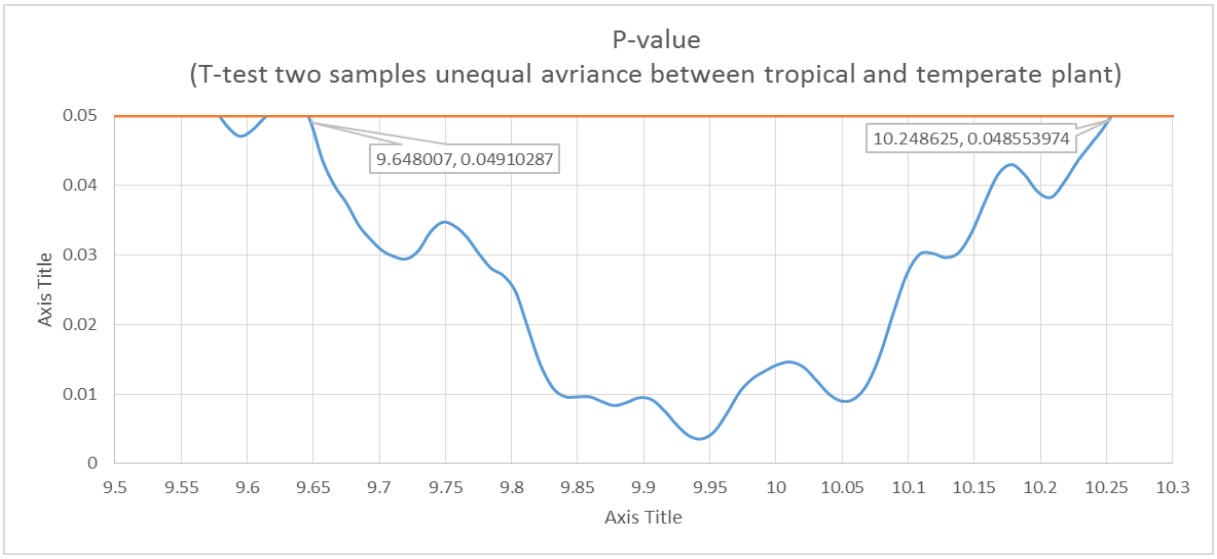

Figure 3-7: P-value of T-test between tropical and temperate plant emissivity, where was less than 0.05

3.3. The correlation coefficient between emissivity and each leaf trait

The correlation coefficient (R value) between leaf trait and emissivity at all (6612) bands and at TIR bands are shown in Figure 3-8 and Figure 3-9 separately. Bottom cuticular membrane was the only one trait, whose absolute R-values was less than 0.5 at all TIR bands. Stomata density was the only trait that had positive R-values at all TIR bands, according to following graphs.

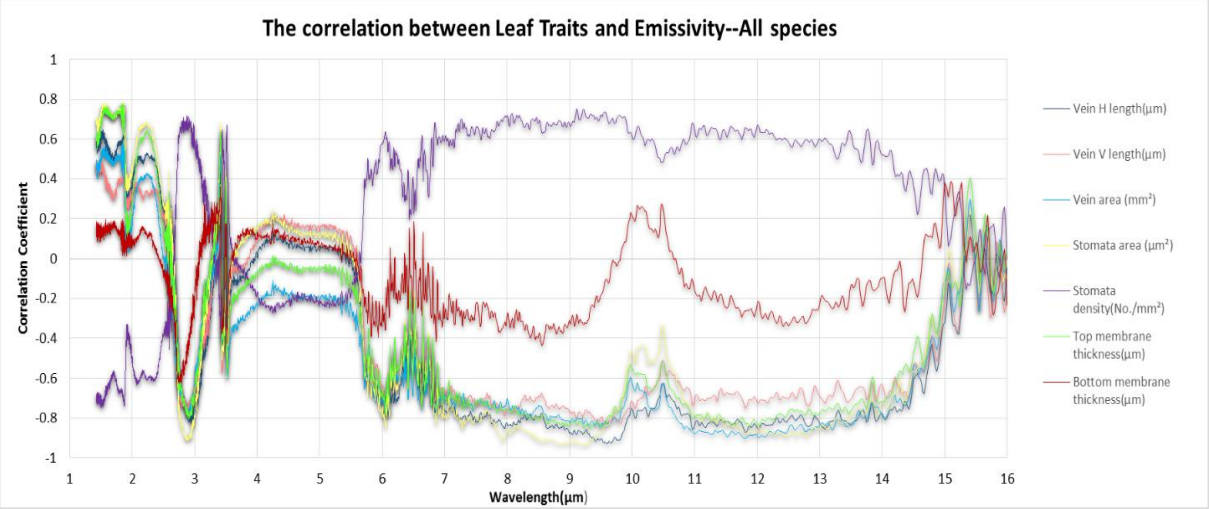

Figure 3-8: R-value of emissivity and each leaf anatomical trait at 6612 bands

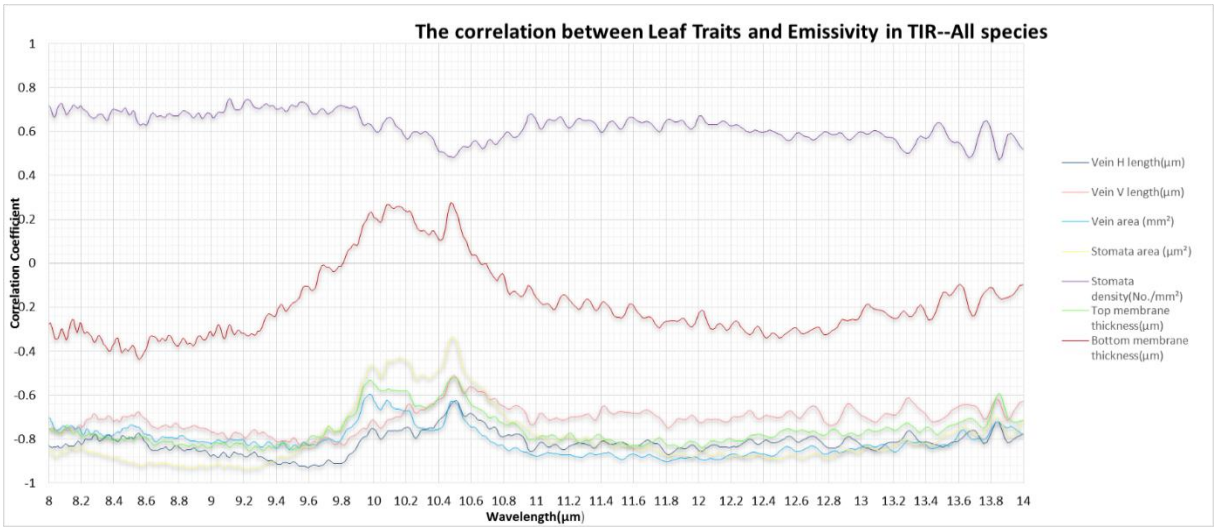

Figure 3-9: R-value of emissivity and each leaf anatomical trait at TIR bands

3.4. Optimal Bands for correlation analysis

The optimal bands were chose from TIR wavelength range (Table 3-4), based on the highest absolute values of correlation coefficient (range from 0 to 1, higher value stands for higher correlation). The leaf traits in rows matched their optimal bands selection. Different anatomical traits had their own optimal bands. Top membrane thickness, for instance, illustrated the highest correlation coefficient (R=-0.858) to emissivity at band 6147 with 9.319μm wavelength. All trait populations were significantly different from emissivity population, with P-values less than 0.001.

Table 3-4: Optimal bands of each leaf traits at TIR wavelength range

Trait name	Band No.	Wavelength (μm)	R-value	P-value
Vein horizontal length	6179	9.594	-0.930	<0.001
Vein vertical length	6180	9.603	-0.828	<0.001
Vein area	6381	11.799	-0.904	<0.001
Stomata area	6136	9.227	-0.934	<0.001
Stomata density	6122	9.114	0.755	<0.001
Top membrane thickness	6147	9.319	-0.858	<0.001
Bottom membrane thickness	6048	8.557	-0.392	<0.001

3.5. Linear regressions for modelling correlation at each traits optimal band

Linear regression was achieved to model the relationship between emissivity and each leaf anatomical trait. All linear models were set at optimal bands for every traits (according to Table 3-4). Coefficient of determination (R squared) was calculated to evaluate the fitness level of these models. Figure 3-5 showed the linear regression equations and R squared results. Every point represented one leaf sample with different colours of indicate species, which regarded as a whole database for the linear regression. From the Figure 3-10, only stomata density had an increased trend with the value of emissivity increasing, while others showed decreased tendency. All the linear models had been test with a high R squared value (large than 0.5).

Form the distribution of points in Figure 3-11, major traits trended to a straight line while stomata density and bottom cuticular membrane thickness did not fit linear model very well. Therefore, other models were tested the fitness of those two traits. Quadratic polynomial and logarithmic equations had a better performance on those two traits with higher R squared values, which were 0.88 and 0.67, respectively (as Figure 3-10 showed). From the stomata area plot, there was an obvious blank in the middle of line, which also could found in Figure 3-1.

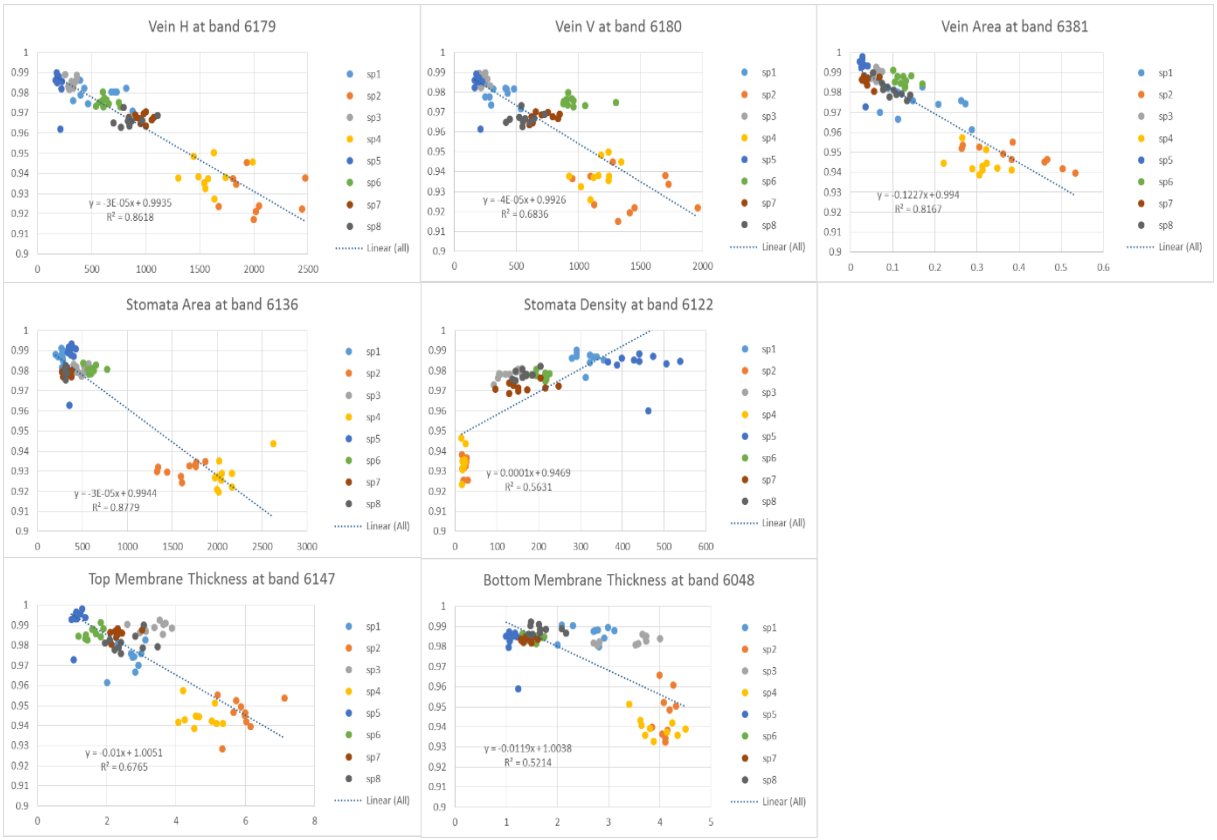

Figure 3-10: Linear correlation between leaf anatomical traits and emissivity at different bands per leaf

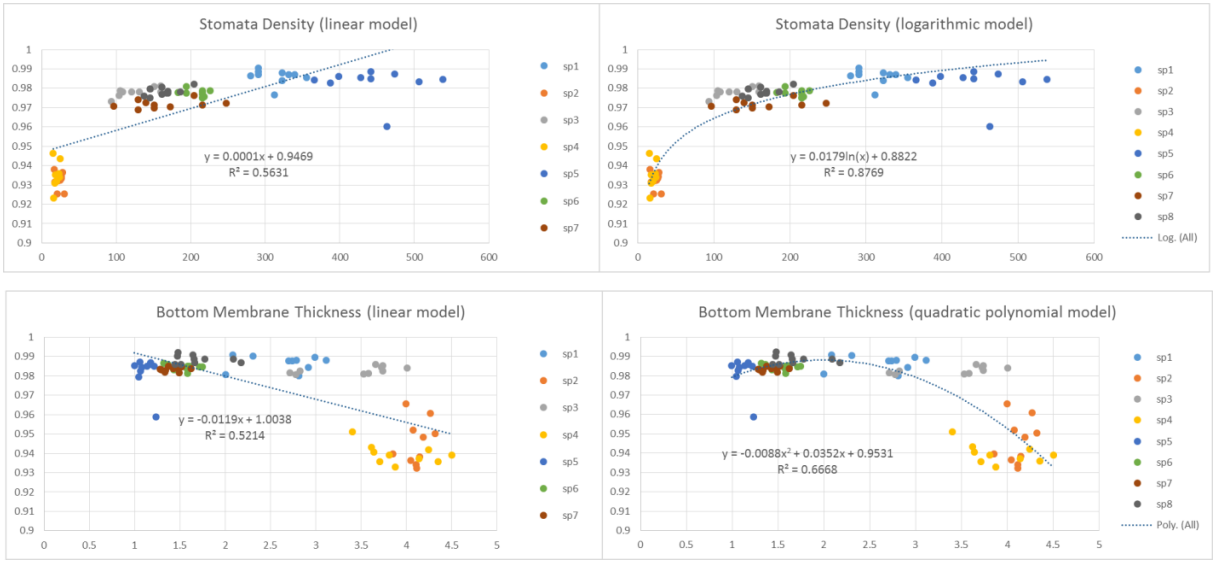

Figure 3-11: Comparison of two models of correlations between emissivity and stomata, and between emissivity and bottom cuticular membrane thickness

Linear regression was also used to model correlation based on averaged species data, which only included 8 points represented for 8 species (Figure 3-12). In the Figure 3-12, red points and grey points represented for the tropical species and temperate species, respectively. The linear regression equations were similar to the previous ones (with the leaf database), each point was averaged from ten leaves. With smaller variations, the values of R squared were higher than the previous Figure 3-10. Figure 3-13 compares the different model fitness for stomata density and bottom cuticular membrane thickness.

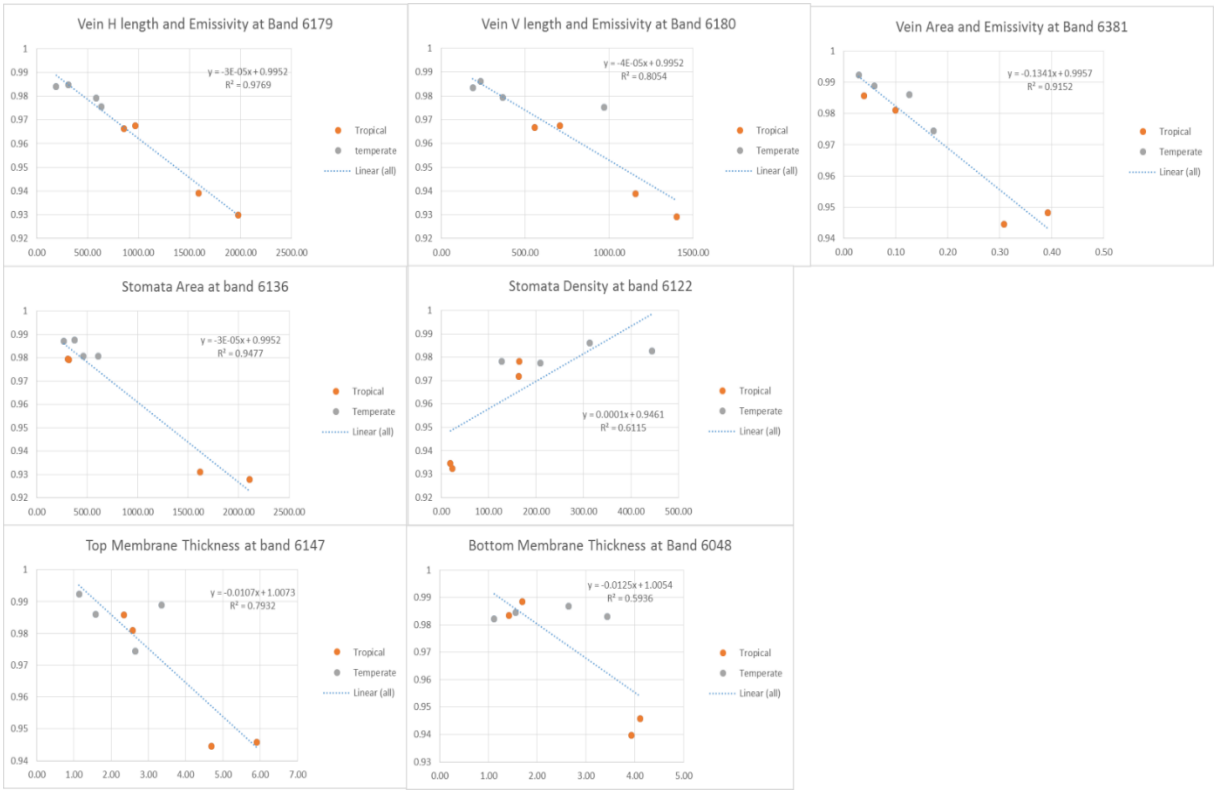

Figure 3-12: Linear correlation between leaf anatomical traits and emissivity at different bands per species

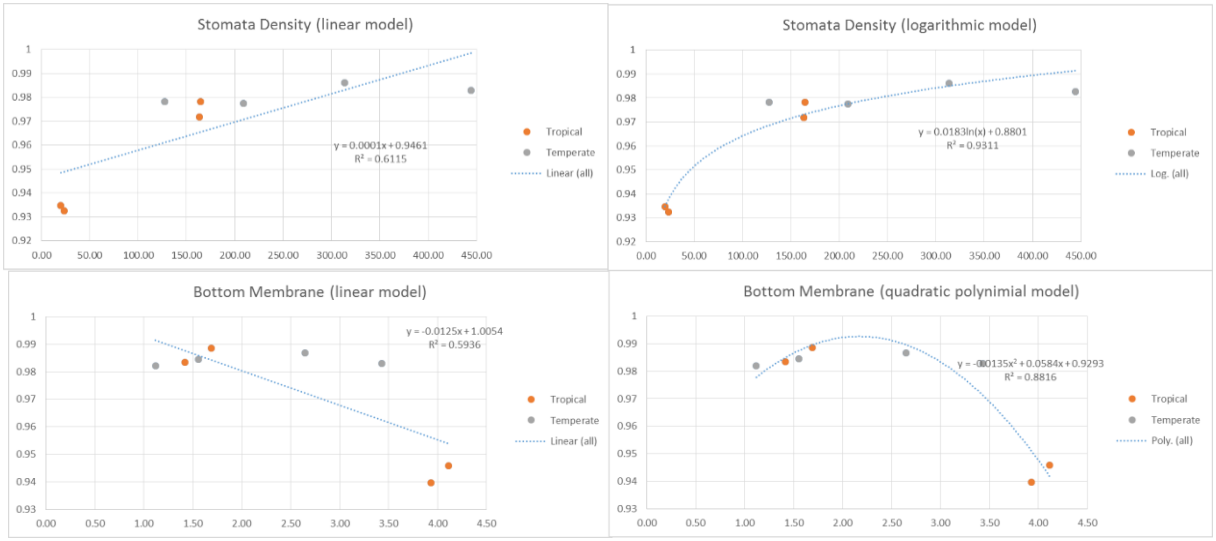

Figure 3-13: Comparison of two models of correlations between emissivity and stomata, and between emissivity and bottom cuticular membrane thickness

3.6. The correlation between each two different leaf anatomical traits

Table 3-5 shows the correlation coefficient between two different traits. Each pair of traits showed strong correlation with each other, except for the pair of vein vertical length and thickness cuticular membrane on the bottom side, which correlation was weak with a low R-value (0.46<0.5). Stomata density was a particular trait which was the only one that showed significantly negative correlation with other anatomical traits, while other traits were positively correlated with each other. Vein horizontal length and vein vertical length, as well as top and bottom membrane thickness showed highest two correlations with 0.89 and 0.90, respectively.

Table 3-5: Correlation coefficient between each two leaf anatomical traits

Trait name	Vein H length (μm)	Vein V length (μm)	Vein area (mm^2)	Stomata area (μm^2)	Stomata density (No./mm^2)	Top membrane thickness(μm)	Bottom membrane thickness(μm)
Vein H length (μm)	1.00	0.89	0.87	0.79	-0.75	0.82	0.61
Vein V length (μm)		1.00	0.80	0.76	-0.69	0.64	**0.46**
Vein area (mm^2)			1.00	0.80	-0.60	0.81	0.73
Stomata area (μm^2)				1.00	-0.69	0.77	0.73
Stomata density (No./mm^2)					1.00	-0.79	-0.69
Top membrane thickness (μm)						1.00	0.90
Bottom membrane thickness (μm)							1.00

4. DISCUSSION

4.1. Independent sample diversity

Every leaf is unique and these unique characteristics not only associate with the genetic variability within a species, the age of a leaf or the location of a plant, but also relates to changing environmental conditions. Figure 3-1, as an example illustrates that between leaves of the same species differences in traits exist. That differences are clear between different species is also shown in Table 3-1. The results suggest that species variability is the dominant factor for explaining differences in leaf features. Furthermore, according to the results of Table 3-2, there are even significant differences of traits between tropical and temperature species. Plants living in tropical areas, for instance, in general have a larger size of veins, probably to supply enough water for intensive transpiration. As Table 3-1 illustrates, Species2 and Species4 have larger veins than the other six species. Species2, for instance, whose horizontal vein diameter is three times as wide as Species1 and in vertical direction is four times wider. However, the vein area of Species2 is only nearly twice as large as Species1. Based on initial microscope observations it appeared that vein structure differed between tropical and temperate plants. For tropical plants, the main vein consisted of many separated vessels, while the temperate plants mostly contained one large central. If we calculated total cross-sectional vessel area, tropical and temperate plants had smaller differences on the area of vein. That would be the reason to explain differences in lengths but similar areas.

Another example is shown in Table 3-3, the total stomata area of temperate plant is more than twice larger than that of tropical plant. Plant adaptation on environmental conditions can provide a reasonable way to answer that observation of stomatal area. From the view of plant physiology, plants have the ability to update their features to survive conditions like hot and dry environments. If a plant gets enough carbon dioxide with fewer stomata, meaning more water can be conserved, that plant would be advantaged over other species (Paleontology, 2008). Tropical plants improve their stomatal efficiency by enlarging single stomata areas and decreasing the density of stomata, to control the intensity of transpiration. In addition, tropical plants thicken their cuticular membrane on the top side (Table 3-1) for the same reason, conserving water.

All the characteristics of a leaf are plant responses to environmental conditions, plants continually adapt themselves for survival under the changing surroundings. Therefore, the various environmental conditions are probably at the root of species variability.

4.2. Emissivity spectrum

Plant emissivity at TIR bands is more responsive to leaf structure properties, while within MIR wavelength region, emissivity spectrum is more responsive to leaf water content (Ribeiro da Luz & Crowley, 2007; Salisbury, 1986; Ullah et al., 2012b; Wong & Blevin, 1967)

This thesis focuses on TIR bands, but a few extraordinary features of the emissivity spectrum have been illustrated by Figure 3-2. In that figure, the emissivity of species1 is significantly lower than other species at MIR bands. There are two possible explanations for that emissivity performance. The first one is because of the unique leaf structure. Species1 is a temperate herbaceous species, but it is quite different from other tested temperate species. It is covered with extremely long and dense trichomes on both sides to resist low temperates in winter. Therefore, trichomes might be an important factor that reduces emissivity in the MIR. Based on the previous results we know that MIR bands are closely related to leaf water content and not to leaf structure. Therefore, the low emissivity of species1 may due to the low water content of trichomes on the leaf top surface. Or the second reasonable explanation, the low emissivity is caused by the early morning dew that attached to leaf trichome. As the leaf spectral measurement operated at the end of September of 2013, when a big temperature change happened between morning and night. The spectral measurement is operated during the morning, when dews might attach to leaf surface, though those sample leaves have been picked and kept in room for one hour before the measurement.

Focusing on the TIR bands (Figure 3-3), the emissivity spectra of species2 and species4 are quite differenc from the other species. That emissivity difference probably arises because of differences in the vein size and the stomata factors (stomata area and stomata density). Species2 and species4 are tropical plants that were growing in the ITC building. They have a larger size but lower density of stomata in common compared to the other species. Besides, these two species have no trichomes on their leaf surfaces, so their leaves are quite smooth. Species7 and species8, are also tropical species, but their traits are closer to temperate plant traits (from Table 3-1), which may be because they also live in sub-tropical area. Therefore, the emissivity spectra of species7 and species8 show a similar trend to temperate plant emissivity.

Compared with tropical plants, temperate plants have higher emissivity as Figure 3-4 and Figure 3-5 shown, but not all TIR wavebands can strongly support the hypothesis (the temperate plant has a higher emissivity than tropical plant), when looking at a t-test (Figure 3-7). If the P-value is less than 0.05, which means the probability of accepting hypothesis is larger than 95%, otherwise, the confidence of rejecting the 0-hypothesis is weak.

4.2.1. Correlation between emissivity and anatomical traits

The correlation between emissivity and each trait was tested, and the results are showed in Figure 3-8 and Figure 3-9. Correlation values close to positive one or negative one, indicates a strong correlation. If the absolute correlation value is less than 0.5, it means the correlation is weak.

Bottom cuticular membrane thickness showed a weak correlation with emissivity, which makes sense, as the emissivity is measured on the top leaf side, so the bottom side probably has little correlation with emissivity. Stomata density, was the only trait of seven anatomical traits, which is positively correlated with thermal emissivity. That means plants with more stomata will lose their heat more easily through long wavelength radiation. Therefore, some temperate plants have many stomata to keep cool. On the other hand, plants living in tropical area usually have fewer stomata, and lower emissivity to keep their leaf warm. Stomata size, vein lengths (diameters of long and short arises), vein area and top cuticular membrane thickness are negatively correlated with emissivity, which means plants that have small stomata, small vein and thin top cuticular membrane will easily to lose heat through thermal wavelength radiation. As one main function of the leaf vein is used for transporting the water for plant transpiration, if plants have larger veins, the intensity of transpiration will be higher with lower emissivity. That is a possible reason to

explain why many temperate plants have higher emissivity with smaller stomata, smaller vein and thinner top cuticular membrane to help temperate plant resist the cold in winter.

4.3. Regression

As Figure 3-10 shows different traits correlate with emissivity following different trends, all the emissivity of species are less than 1. That is because the "emissivity of a surface is the ratio of its emissive power to that of a black body for a given wavelength and at the same temperature"(Dictionary). Black body is an ideal object which can emit all its' energy. Therefore the emissivity of black body is 1, while any real object emissivity is less than 1.

For some traits, such as vein diameters, vein area and stomata density, the correlations between the traits and emissivity are formed by a straight line. While the stomata density and the bottom cuticular membrane thickness show a curve trend of correlation. Therefore, I tested the regression with different models based on the tendency of correlation. Linear regression is testing stomata density, vein lengths and vein area. Quadratic polynomial and logarithmic equations are used for testing stomata density and bottom cuticular membrane correlation with emissivity. However, the results only suggest the correlating tendency between emissivity and traits, which cannot show a causal relationship between them. For stomatal area, the points seem organized into two separate groups, and it is therefore hard to suggest the correlation will be a straight line or follows other curves.

Figure 3-12 shows average leaf traits per species, which was divided over temperate and tropical species, helping to show the distribution range difference between tropical and temperate species. That figure suggests temperate plants have a wider distribution in trait values than tropical plants, probably because some temperate species also occur in sub-tropical area. Furthermore, even in some tropical areas, some temperate species can still survive.

4.4. Correlation between each two different traits

Leaf anatomical traits may correlate with each other, so their correlation is tested by R-value, which results are organized by a table (Table 3-5). Table 3-5 explains the similarity of correlations between emissivity and leaf traits from another view. In Table 3-5, for example, stomata density negatively correlates with other traits, supporting the result of correlation with emissivity (opposite to other traits' correlations with emissivity).

5. CONCLUSION

In this thesis, I measured leaf emissivity and seven leaf anatomical traits. All the measurements were based on controlling good growing conditions for plant samples. Therefore, if the plant would have been sampled under dry, cold or other extremely conditions, the results might be different.

According to the results, I find that when plants change their leaf traits, their emissivity also changes. Six of the anatomical traits (except for cuticular membrane on leaf bottom side) correlate with emissivity at TIR bands, especially in the 8-10μm and 11-13μm wavelength range. Stomata density was the only trait showing a positive correlation with emissivity, while the other five had negative correlations with emissivity. Some anatomical traits illustrate similar correlations with emissivity (for example, vein horizontal and vertical diameters), probably because these traits themselves correlate with each other. Thus, it is hard to conclude which trait had most effect on emissivity. The anatomical variations between tropical and temperate plants show differences, suggesting these variations show the plants adaptation to different conditions. While within some range, those traits are overlapping, probably due to the fact that these plants are capable of living under similar conditions. It is known that transpiration correlates with leaf water content. The results from this experiment suggest that if the plant is growing under good conditions (such as, enough water supplied and enough solar energy), then the plant transpiration probably has a strong effect on emissivity. But if the plants live in limited conditions (such as dry or cold places), the emissivity probably depends on the water content of the leaf.

For future studies, trichome traits may be tested, provided suitable instruments for measuring these is available, because trichomes attaching to the leaf top surface probably affect the emissivity. Also a future study could focus on one trait (given the string correlations between them) but testing more samples.

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