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INDUCED EM-SPECTRAL RESPONSE FOR AN EARLY DETECTION OF VEGETATION (CROP) BIOTIC STRESS Natural Science and Natural Heritage

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ABSTRACT

All vegetation is vulnerable to stress due to biotic or abiotic factors. Remote sensing technology is a useful tool in managing abiotic stress. However, it is limited to damage assessment for biotic stress. The objectives of this study are to determine the pre-symptom stage of ralstonia solanacearum infection in cucumber and to study the effects of nutrient content, water level and light exposure on the electromagnetic spectral response of cucumber leaves in the pre-symptom stage. In this study, hydroponic sets of inoculated and non-inoculated cucumber plants were grown in a controlled environment. A set of each inoculated and non-inoculated cucumber were subject to nutrient stress, water stress and light stress. Spectroradiometer measurements, photographs and notes were collected daily. Mean percent difference between inoculated and non-inoculated cucumber in normal condition of less than 15% indicates that data was collected during the pre-symptom stage of ralstonia infection. From the five vegetation indices used, the NRI, TVI and MCARI were successful in detecting a change in spectral reflectance in infected cucumber leaves that has been induced by light stress during the pre-symptom stage. This study has proven the hypothesis that a measurable change in spectral response can be induced for the early detection of vegetation biotic stress.

1. INTRODUCTION

Background of study

All vegetation is vulnerable to stress due to biotic or abiotic factors. According to Nilsen & Ocrutt (1996), biotic stress are caused by competition, allelopathy, herbivory, diseases, pathogens and viruses; while abiotic stress are caused by extreme physical or chemical alterations. According to Pawar (2009), about one fourth of global agricultural produce is lost due to biotic stress. Therefore, an effective system is needed to detect and manage biotic crop stress in order to reduce yield loss.

Conventional biotic stress detection involves going out into the field and visually assessing the signs and symptoms of disease or pest in the crops. This method has high cost of manpower and time, besides poor spatial coverage. As a result, pesticides and fertilizers were often applied evenly over the whole field when it may only be needed in a small part of the field. Therefore remote sensing can be a useful tool for precision farming.

For agriculture application, remote sensing is commonly used to classify crop type, crop area, predicting crop yield, soil survey, irrigation planning, and damage assessment by disaster, pest or diseases. It is widely used in managing abiotic stress such as nitrogen deficiency and water stress in order to improve crop yield. However, when it comes to biotic stress, remote sensing is currently only able to assess the damage of diseases through the use of vegetation indices (Apan et al., 2004; Devadas et al., 2008). Remote sensing is not yet useful in preventing losses due to biotic stress.

Therefore, further research is needed to study the early detection of biotic stress in plants. However, very little progress has been made to achieve early detection of biotic stress using remote sensing. In this study, this problem will be addressed by studying how a measurable change in spectral signature of a diseased plant in its pre-symptomatic stage can be induced through the manipulation of standard farming practices or abiotic factors. By inducing a change in the diseased plant, the measured electromagnetic signature would contain new information that cannot be measured using conventional remote sensing method.

In this study, two sets of cucumber plants (*Cucumis Sativus*) was grown in a controlled environment where one set is inoculated with bacterial wilt (*Ralstonia Solanacearum*) and another set is the experiment control. During the pre-symptomatic stage, both sets of cucumber plants was subjected to changes that can be applied in the field such as nutrient content, water content and light exposure. Spectral reflectance of the cucumber was measured using a spectroradiometer.

Problem statement

Remote sensing instruments measure electromagnetic radiation that is reflected or emitted from an object of interest. If two different objects reflect the same electromagnetic energy, these two objects would appear the same in a remotely sensed image. Therefore, remote sensing cannot detect any phenomena that are not shown by a measurable change in reflected electromagnetic energy.

When a plant is infected by bacteria, virus or fungi, there is a period of time during which there are no visible symptoms of infection on the plant. This is usually because the plant has resistance mechanism against infection or the pathogen takes time to develop within the plant. In most cases, symptoms develop only when the infection is in its advanced stage. When this happens, the plant must generally be destroyed in order to prevent further spreading.

Conventional technique of remote sensing is not able to detect infection of plants before symptoms become visible. At this point, the damage is already done and treatment of the infection is not effective. Therefore, a technique for the early detection of vegetation biotic stress is needed.

Objectives

The objectives for this study are as stated below:

- 1. To prove the occurrence of pre-symptom stage of pathogen infection in cucumber plants.
- 2. To determine the possibility of inducing a measurable spectral change by using nutrient, light exposure and water stress during the pre-symptom stage

Significance

Vegetables are vital in sustaining the global human population. Although the world production of vegetables has increased almost three-fold over the last 30 years (FAO, 2006), hunger and starvation still affects most of the third world countries. According to Pawar (2009), about one fourth of global agricultural produce is lost due to biotic stress. Therefore, solutions are needed to prevent unnecessary losses of crop yield due to biotic stress.

The early detection of plant biotic stress would give farm managers information on the occurrence of disease in crops before symptoms develop. If treatment (fertilizers or pesticides) are administered during the pre-symptom stage, huge losses could be prevented. 5

This study would validate the early detection of cash crop diseases through the synchronization of remote sensing data acquisition with routine farming practices and environmental fluctuations. Therefore, the result of this study serves as a significant stepping stone towards the implementation of this technique in precision farming practices.

3. RESEARCH METHODOLOGY

Introduction

This chapter reviews the process of studying the effects of conventional farming practices and abiotic parameters on the plant electromagnetic spectral signature in the prephysiological disease stage. Therefore, a few experiment activities are needed to be carried out to achieve the objectives of this study. In this chapter, the methodology, experiment setup, data collection, data processing and the analysis of the data are reviewed.

Methodology

The methodology of this study can be divided into four main steps which are experiment setup, data collection, data processing and data analysis. Each step plays an important role in leading the study to achieve its objectives successfully. Therefore, each step needs to be carefully executed in order to achieve results with minimal ambiguity. Eight sets of hydroponically grown cucumber plants were grown in a controlled environment. These sets consisted of combinations of normal cucumber, inoculated cucumber, optimal conditions, manipulated nutrient content, manipulated water level, and manipulated light exposure.

During a ten day period, the spectral signatures of the eight sets of cucumber were taken using handheld spectroradiometer in artificial lighting on a daily basis. At the same time, photograph of the cucumber sets and notes were taken. The photographs and accompanying notes would aid in the understanding of the spectroradiometer measurements and analysis.

From the daily spectral signature measurement, the pre-symptom stage of the disease in cucumber was determined. The pre-symptom stage is the duration of time between inoculation and symptom development. This stage is when the spectral signature of healthy plant and inoculated plant is similar. The hypothesis of this experiment is that there would be a detectable change in spectral signature in the sets that has abiotic manipulation (e.g. nutrient, water and light exposure) during this pre-symptomatic stage.

Finally, the spectral measurements were quantitatively compared using a combination of vegetation indices such as the Normalized Difference Vegetation Index, Structure Insensitive Pigment Index, Nitrogen Reflectance Index, Triangular Vegetation Index and Modified Chlorophyll Absorption and Reflectance Index. Using the vegetation indices, the difference in spectral signature was quantified. The flow of methodology is described in Figure 3.1.



Figure 3.1: Flow chart of methodology.

Experiment Setup

In order to conduct the experiment successfully, several factors need to be controlled. The experiment setup includes a controlled environment, protocol for growing cucumber, inoculation of the cucumber and abiotic factor manipulation.

Controlled Environment

A controlled environment is where a set of environmental factors such as temperature, humidity and lighting are regulated. The controlled environment was set in a greenhouse which provides protection from pests and the environment. The temperature and light radiation of the greenhouse was regulated by a black net which diffuses the sunlight as it enters the greenhouse. The exhaust fan was used to regulate humidity and promote air circulation in the controlled environment. The greenhouse and all facilities were provided by Taman Pertanian Universiti, Universiti Putra Malaysia.



Figure 3.2: The greenhouse used at Taman Pertanian Universiti, Universiti Putra Malaysia.

Growing Cucumber

The cucumber seeds were placed in slits made in a sponge which was kept damp with water mixed with fertilizer (Figure 3.3(a)). On the third week, the cucumber seedlings were transplanted to eight separate hydroponic sets (Figure 27 3.3(b)). The hydroponic sets were separated from each other to prevent contact between the different sets of cucumber. On the seventh week (Figure 3.3(c)), four of the hydroponic sets were inoculated with *ralstonia solanacearum*.





Figure 3.3: (a) One week old cucumber growing in damp sponge. (b) Three weeks old cucumber seedlings after being transplanted to hydroponic set. (c) Seven weeks old cucumber plants before inoculation.

Inoculation

When the cucumber seedlings are 7 weeks old, 4 out of the eight sets would be inoculated with a pathogen. For this study, the bacteria *ralstonia solanacearum* was used. This bacterium is commonly known as bacterial wilt and it infects a wide range of vegetables and plants. The cucumber plants were inoculated with the bacteria by creating lacerations at the root and adding the cultured bacteria into the hydroponic solution (Figure 3.4 (a) & (b)).



Figure 3.4: (a) Creating laceration at roots of cucumber plant using a scalpel. (b) Adding cultured bacteria to hydroponic solution.

Manipulated Parameters

Several parameters were manipulated in order to induce a change in spectral signature in the inoculated cucumber in the pre-symptom stage. These parameters simulate certain farming practices and environmental fluctuations so that the induced spectral signature method can be applied for early detection of crop biotic stress in precision farming. In this study, the parameters that were manipulated were the level of hydroponic water, amount of light radiation, and nutrient concentration. The selection of parameters to be manipulated was done by taking into consideration the feasibility of execution in the controlled environment and available resources.

Manipulated water level

One set of healthy cucumber and inoculated cucumber had manipulated water level. In farming practices, the amount of water available to the crop is affected by both the weather and man through precipitation and irrigation. Therefore, the water level in the hydroponic set was reduced to simulate drought.

Manipulated light radiation

One set of healthy cucumber and inoculated cucumber had manipulated light radiation. Of all the environmental fluctuations, the change of daylight intensity is the most reliable. In the morning, light radiation increases as the sun rises, peaks in the afternoon, and decreases as the sun sets in the evening. Therefore, the light radiation was manipulated by covering the cucumber plant for an extended period of time.

Manipulated nutrient

One set of healthy cucumber and inoculated cucumber had manipulated nutrient content. The main components of common fertilizers are nitrogen (N), phosphorus (P) and

potassium (K). The application of fertilizer is a ubiquitous farming practice in modern day agriculture. It is one of the factors that can be almost entirely manipulated by man. Therefore in this experiment, the hydroponic solution used contained little or no fertilizer.

Data Collection

Data Collecting Equipment

For the purpose of this study, the Analytical Spectral Devices (ASD) FieldSpecPro Spectroradiometer was used. The spectroradiometer can measure spectral response within the range of 350 nm to 2500 nm at 1 nm intervals. The spectroratiometer was operated using a Panasonic Toughbook Model CF-29. The spectroradiometer was attached to a pistol grip with an 8° foreoptic.



Figure 3.5: (a) ASD FieldSpecPro Spectroradiometer connected to the Panasonic Toughbook. (b) Fiberoptic attached to pistol grip with 8° foreoptic.

Spectroradiometer Measurement

The spectral signature of the cucumber was measured using ASD FieldSpecPro spectroradiometer. The geometry of spectroradiometer measurement was kept constant to prevent errors due to different look and lighting angles. This was achieved by using artificial light fixture as source of radiation. The light source, target (cucumber leaf) and the spectroradiometer lie on the same plane. The look angle, θ was kept constant. The geometry of spectroradiometer measurement is shown in Figure 3.2.



Figure 3.6: Geometry of spectroradiometer measurement with respect to target and light source.

Before taking spectroradiometer measurements, dark current was first taken to calibrate the spectroradiometer and white reference to reduce effects of light source spectral signature. During spectroradiometer measurement, ten spectrum saves was collected with one second interval to reduce random error in measurement.

Ancillary data

Besides taking spectroradiometer measurements, notes were taken based on human observation of the lettuce. The notes contained information such as date and time of measurement, age of lettuce, plant height, maximum leaf width and other observations.

Additionally, photographs were taken of the cucumber during spectroradiometer measurement. These photographs give visual support to spectroradiometer measurements and the notes taken. The photographs and accompanying notes aid in relating the spectroradiometer measurements to what is actually seen on the lettuce.

Processing and Analysis

Once the spectroradiometer measurement has been taken, the ten spectrum saves of each measurement was averaged using the Mean function in ASD ViewSpecPro and exported to MS Excel where the pre-symptom stage and induced change in spectral response was determined. The mean spectral data are given in Appendix B.

Determine Pre-symptom Stage

The pre-symptom stage is the duration of time between inoculation and symptom development when the spectral signature of healthy plant and inoculated plant is similar. The hypothesis of this experiment is that there would be a detectable change in spectral signature in the sets that has abiotic manipulation (e.g. nutrient, water and light exposure) during this pre-symptomatic stage. So, to prove this hypothesis, the data must be collected during the pre-symptom stage of infection. The mean percent difference is used to determine the pre-symptom stage. The process used is described in Equation 1 below.

Mean Percent Difference =
$$\sum_{R_H} \left(\frac{|R_H - R_I|}{R_H} \right) \times \frac{100}{N}$$

where, RH and RI are the reflectance values of non-inoculated and inoculated cucumber and N is the number of wavelength bands. The pre-symptomatic stage of the disease is assumed to have developed into symptomatic stage when the percent difference in reflectance is more than 20%.

Vegetation Index

From literature review, several studies comparing the effectiveness of vegetation indices for disease detection were reviewed. From these studies, several vegetation indices were found useful in detecting change in pigmentation or discriminating diseases in certain crops. Therefore, the vegetation indices used in this experiment are the Normalized Difference Vegetation Index, Structure Insensitive Pigment Index, Triangular Vegetation Index, Nitrogen Reflectance Index and Modified Chlorophyll Absorption and Reflectance Index.

Although previous studies have found that the Normalized Difference Vegetation Index, NDVI is not effective in disease discrimination, this vegetation index shall be used in this experiment as it is still one of the most widely used vegetation index. The NDVI was applied as in Equation [3.2].

$$NDVI = \frac{R_{850} - R_{680}}{R_{850} + R_{680}}$$

where, R850 and R680 are the reflectance value at wavelengths 850 nm and 680 nm respectively. The possible range of NDVI values is -1 to 1 where NDVI values above zero represent vegetation with photosynthetic activity while negative NDVI values represent non-vegetative objects or dead vegetation. Higher NDVI values are caused by increased NIR reflectance and increased red chlorophyll absorption.

The Nitrogen Reflectance Index, NRI is used to estimate nitrogen content in the target which is related to the chlorophyll-*a* content. The NRI is given in Equation [3.3] below.

$$NRI = \frac{R_{570} - R_{670}}{R_{570} + R_{670}}$$

where, R570 and R670 are the reflectance value at wavelengths 570 nm and 670 nm respectively. Possible range of values for NRI is -1 to 1. Increased NRI values are caused by increased green reflectance and increased red chlorophyll absorption. The chlorophyll absorption can be used to estimate nitrogen content in the plant.

The Structure Insensitive Pigment Index, SIPI is another commonly used vegetation index. It is mainly used to estimate the ratio of carotenoids to chlorophyll-*a*. The SIPI can be applied as in Equation [3.4].

$$SIPI = \frac{R_{800} - R_{445}}{R_{800} + R_{680}}$$

where, R800, R445 and R680 are the reflectance value at wavelengths 800 nm, 445nm and 680 nm respectively. The SIPI values for vegetation always approaches 1. When SIPI value is above 1, this indicates that the blue absorption of carotene is higher compared to the red absorption of chlorophyll. When the SIPI value is below 1, the blue absorption of carotene is lower compared to the red absorption of chlorophyll.

The Triangular Vegetation Index, TVI is a relatively new vegetation index which is based on the idea that the total area of the triangle formed by green, red and infra-red bands would give information on chlorophyll absorption and leaf tissue abundance. The TVI is given in Equation [3.5] below.

$$TVI = 0.5[120(R_{750} - R_{550}) - 200(R_{670} - R_{550})]$$

where, R750, R550 and R650 are reflectance values at wavelength 750 nm, 550 nm and 670 nm respectively. The TVI value represents the area formed by the green reflectance peak, the red absorption band and the NIR band. Therefore, the possible TVI value range is always positive. Increased TVI values indicate increased red absorption and increased NIR reflectance which represents leaf tissue abundance.

Besides TVI, the Modified Chlorophyll Absorption and Reflectance Index, MCARI is another vegetation index based on three discrete bands. The MCARI measures the chlorophyll absorption (670 nm) relative to the green reflectance peak (550 nm) and infrared reflectance (700nm) as shown in Equation [3.6].

$$MCARI = [(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})](\frac{R_{700}}{R_{670}})$$

where, R750, R550 and R650 are reflectance values at wavelength 750 nm, 550 nm and 670 nm respectively. Increased MCARI values indicate increased NIR reflectance of leaf tissue abundance, increased red chlorophyll absorption and increased green reflectance while lower MCARI values are due to lower NIR reflectance, less chlorophyll absorption and decreased green reflectance.

Percent Difference in Vegetation Index Values

To quantify the induced change in spectral reflectance between inoculated and noninoculated in a manipulated condition, the percent difference of vegetation index values is used.

% Difference VI =
$$\frac{|VI_{I,x} - VI_{H,x}|}{VI_{max}} \times 100$$

where, VII,x and VIH,x are the vegetation index values for inoculated cucumber and noninoculated cucumber in condition x where condition x may be nutrient stress, water stress or light stress, and VImax is the maximum vegetation index value.

4. LITERATURE REVIEW

Introduction

This chapter serves as a review on research or studies that have been made on the detection of vegetation biotic stress for various pathogens and vegetation species, in order to synthesize the arguments of other researchers. In addition, the use of remote sensing in detecting vegetation biotic stress will be discussed, focusing on various vegetation indices and the more robust hyperspectral vegetation indices. Recent research on the early detection of vegetation biotic stress during the pre-symptom period will also be reviewed.

Besides that, the characteristics, farming practices and pathogens of cucumber will be briefly discussed in order to gain a better understanding on the crop used in this study.

Vegetation Stress

Stress is the reaction of a biological system to extreme environmental factors that may cause significant changes in the system affected (Godbold, 1998). As shown in Figure 2.1, the stress factor can be either biotic or abiotic. The following subtopic defines biotic stress and the general symptoms of biotic stress.



Figure 2.1: The sources of environmental stress in plants (adapted from: Nilsen & Orcutt, 1996).

Vegetation Biotic Stress

According to Mandre (2002), biotic stress is concerned with the interaction between populations, while abiotic stress may be physical or chemical in character. Biotic stress is caused by diseases, herbivores and other plants. All vegetation types are vulnerable to biotic stress. Unfortunately, in the case of agriculture crops, the disease is more easily spread because of the small gaps between plants. Plant diseases are one of the main causes of yield loss and reduced quality. According to Pawar (2009), global yield loss due to biotic stress account for about 25% of the value of agricultural produce. Therefore, it is important to develop an effective system to manage and control biotic stress. The method most commonly used in the management of biotic stress in crops is to spray pesticides evenly over the fields at different stages of the cultivation cycle. However, disease and pest infestation are usually not distributed uniformly over the field but occur in patches. The spraying of pesticides should be focused only on infected patches of the field.

Most vegetation biotic stress affects the supply, distribution or release of water in the plant. This condition may cause the stomata of the leaves to close, disrupt photosynthesis, reduce evapotranspiration and increase the leaf surface temperature. Other symptoms may include physical changes in the plant such as leaf curling, wilting, chlorosis, necrosis or abscission of plant parts (Nilsson, 1995). Although such symptoms can be observed visually, it may be difficult to spot early symptoms of an infection, let alone to quantify them accurately, precisely and quickly.

While discussing about the responses of plants to biotic stress, it is important to note that different species of plants may react differently to the same stress. On the other hand, the plants may have similar reactions to different conditions. Besides that, the response and sensitivity of a plant may vary with age and development stage.

Detection of Vegetation Biotic Stress

Traditionally, a visual approach was used for disease and pest damage assessment. This approach relied heavily on the human eye and brain to assess the occurence of disease or pest in crops. A trained person can assess the visual information available and then concentrate on specific disease symptoms observed. However, this approach requires years of knowledge and experience, is time consuming and labour intensive. Besides that, two different individuals may have different perception, thus resulting in wrong diagnosis of the disease. Therefore, remote sensing method offers a more objective and quantitative way to detecting biotic stress.

Remote Sensing for Detection of Vegetation Biotic Stress

According to Jensen (2000), remote sensing can be defined as an art and science of gathering information about an object without coming into physical contact with it. Thus, it is a useful technique for visualizing, diagnosing, and quantifying vegetation stress. Here, the general approaches used in remote sensing of vegetation biotic stress are briefly introduced.

Vegetation biotic stress can be detected using three types of remote sensing which are thermography, fluorescence and reflectance (Jones, 2008). According to Jones (2008), thermography or thermal sensing is used to study water stress and transpiration. As discussed in a previous topic, stomata closure would reduce evapotranspiration and thus, increase leaf temperature. This change in temperature is the phenomena observed in thermal sensing. According to Chaerle et al. (2002a), fluorescence imaging is used to monitor photosynthetic efficiency of the plant. Fluorescence refers to the emission from a healthy leaf when excited by UV-A radiation which consists of blue (440 nm), green (520 nm), red (690 nm) and far red (740 nm) wavelengths. Fluorescence emissions in red and far red wavelengths are associated with photosynthetic process while blue and green wavelengths are associated with other compounds in the leaf. In reflectance imaging, the electromagnetic signal reflected off the plant leaves is the recorded data. The parameter monitored in reflectance imaging for plant biotic stress is the change in leaf constituents.

As discussed in previous topics, biotic stress causes physiological stress and physical changes in plants. These changes can alter the spectral reflectance signature of the plant. The reflectance of green healthy plants is relatively low in the visible portion of the electromagnetic spectrum due to strong absorption by chlorophyll in their leaves. If the chlorophyll content is reduced due to pests or diseases, the reflectance in the visible region will increase. On the other hand, the reflection of healthy vegetation in the near-infrared (NIR) portion of the electromagnetic spectrum is high. If disease or pests damage the leaves, the reflectance in the NIR region would be lower. In the shortwave infrared (SWIR) region, the spectral signature of healthy vegetation is dominated by water absorption bands. Thus, dry vegetation would have higher SWIR reflectance.

The two general classes of reflectance sensors are multispectral sensors and hyperspectral sensors. Multispectral sensors collect reflected or emitted energy from an object of interest in several bands of the electromagnetic spectrum (Jensen, 2000). An example of a multispectral sensor is the QuickBird high resolution satellite. It has a panchromatic band (450-900 nm), blue band (450-520 nm), green band (520-600 nm), red band (630-690 nm) and near infrared band (760-900 nm).

While multispectral sensors typically collect spectral reflectance with about 2 to 10 wavebands, hyperspectral sensors collect spectral reflectance 10 to hundreds of 13 very narrow wavebands (Lamb et al., 2000). According to Laudien et al. (2004), these narrow bands of the hyperspectral sensors are able to characterize leaf reflectance more precisely compared to the averaged multispectral bands. An example of a hyperspectral sensor is the Airborne Visible Infrared Imaging Spectrometer (AVIRIS) which measures spectral reflectance between 400 nm to 2500 nm using 224 spectral bands with a spectral interval of 10 nm each (Green, 1994). In order to extract vegetation biophysical information from multispectral or hyperspectral images, vegetation indices are commonly used.

Vegetation Indices

Vegetation indices are dimensionless, radiometric values that are designed to assess the spectral contribution of vegetation to spectral observations (Gitelson & Merzlyak, 1996). Until today, there are more than 50 vegetation indices that have been proposed and studied for various applications such as identifying, quantifying, or discriminating biotic and abiotic stresses. There is no one vegetation index that is effective in discriminating between all combinations of healthy plants and pathogen infected plants. Three studies that have been made to test the suitability of a range of vegetation indices for discriminating between a healthy crop and an infected crop would be discussed in this topic.

Genc et al. (2008) evaluated 19 chlorophyll and plant stress related vegetation indices to detect sunn pest density on wheat. A Portable FieldSpec Handheld Spectroradiometer was used in a field experiment at a farm in Turkey. Regression analysis was used to determine the relationship between vegetation indices and initial number of sunn pest. It was found that 4 out of the 19 vegetation 14 indices used were useful in detecting sunn pest density on wheat. The four vegetation indices (Table 2.1) were derived from blue, red and NIR wavelengths.

	Equation	Sources
Normalized Difference	(R750-	Sims and Gamon (2002)
Vegetation Index – 1,	R705)/(R750+R705)	
NDVI-1		
Normalized Difference	(R801-	Daughtry et al. (2000)
Vegetation Index -2 ,	R670)/(R801+R670)	
NDVI-2		
Normalized Difference	(R789-	Rouse et al. (1973)
Vegetation Index – 3,	R649)/(R789+R649)	
NDVI-3		
Structure Insensitive	(R800 - R445)/(R800	Penuelas et al. (1995)
Pigment Index, SIPI	-R680)	

Table 2.1: Vegetation indices used to detect sunn pest disease on wheat. (source: Genc et. al., 2008)

 Vegetation Index

Broge & Leblanc (2000) studied the stability of recently proposed vegetation indices using a combination of two canopy reflectance models, PROSPECT and SAIL (Scattering by Arbitrarily Inclined Leaves). The vegetation indices were compared with respect to leaf area index and chlorophyll density. The sensitivity of the vegetation indices to canopy architecture, illumination geometry, soil background reflectance and atmospheric conditions were analyzed. The Triangular Vegetation Index, TVI was developed as part of this study. The TVI represents the area of the triangle formed by the green peak, chlorophyll absorption minimum and the near infrared shoulder in spectral space. Canopy reflectance was simulated using canopy reflectance model and no ground data was used. So, real world application of the vegetation index should be further validated.

Daughtry et al. (2000) proposed a strategy for detecting leaf chlorophyll status in plants by simulating canopy reflectance using the SAIL model and selecting 15 wavelengths that are sensitive to leaf chlorophyll concentration. As part of this study, Daughtry et al. (2000) altered the Chlorophyll Absorption Ratio Index (CARI) to produce the Modified Chlorophyll Absorption Ratio Index, MCARI. The MCARI uses the green, red and near infrared bands which has been proven to be sensitive to chlorophyll differences in vegetation. Canopy reflectance was simulated using canopy reflectance model and no ground data was used. So, real world application of the vegetation index should be further validated

Diker and Bausch (1998) used data collected from experimental maize plots to develop relationships between reflectance data and several crop parameters and successfully used the Nitrogen Reflectance Index, NRI to estimate leaf chlorophyll content and to predict maize harvest yield. Although there is a clear correlation between the NRI and leaf chlorophyll content, the main interest of the research was to use the NRI for yield forecasting.

Delalieux et al. (2009) evaluated all possible two-band and derivative two-band combination between 350 nm and 2500 nm to assess their accuracy in tracking leaf spectral change caused by apple scab. Apple plants were grown in a controlled environment. There were three sets of apple plants – infected, mock-infected and control. The spectral reflectance of leaves was measured using spectroradiometer. Assessment of vegetation indices was done using Discriminatory Performance (c-index). It was found that ratio indices of wavelengths 1500nm and 2250 nm are most efficient for early detection of apple scab due to the moisture sensitivity of the two bands. Besides that, ratio indices R440/R690 and R695/R760 were useful in discriminating infected apple plants at more developed infection stage. In this study, Delalieux et al. (2009) took the phonological stage of the leaves into consideration. Thus, the results of this study are less ambiguous as the previous two studies. However, only ratio indices, standardized difference vegetation indices and derivative indices are used. Furthermore, the scope of this study is limited to the detection of apple scab. Therefore, the results of this study may not be applicable to other vegetation types.

Early Detection of Vegetation Biotic Stress

The early detection of biotic stress in cash crops is an important advancement in remote sensing for crop management. Chaerle et al. (1999) was able to detect spots of elevated temperature that were confined to area of Tobacco Mosaic Virus (TMV) infection on tobacco leaves before any disease symptoms became visible using infrared images. Plant leaves have stomata which regulate transpirational water loss, which closes in the case of water shortage, thus reducing transpirational cooling. Another mechanism that closes the stomata is salicylic acid, a main compound in the plant's defense against pathogens. The local increase in temperature can be detected though thermography. In addition to transpiration, chlorophyll fluorescence is physiological parameter. Chaerle et al. (2002b) stated that, "If the amount of light absorbed by chlorophyll exceeds the capacity of the photosynthetic chain, the surplus is dissipated as light of longer wavelength. This phenomenon is known as chlorophyll fluorescence. If a plant leaf is affected by stress, photosynthesis is impaired resulting in a bigger share of non-utilized light energy emitted as fluorescence". Therefore, both thermal and fluorescence imaging was combined to monitor spontaneous cell death in tobacco.

This method has its advantages and limitations. Thermography is a passive system, thus it would not affect the target of interest. However, it is highly sensitive to environmental changes such as weather and moisture. Chlorophyll fluorescence imaging enables the

quantification of photosynthetic parameters. However, this method requires a light source of known intensity or artificial illumination. Besides that, the research conducted by Chaerle et al. (1999 and 2002b) were limited to TMV of tobacco plants. Thus, the application of the results of that research on other crop species remains debatable.

Cucumber (Cucumis Sativus L.)

Cucumber (*Cucumis Sativus L*.) belongs to the Cucurbitaceae family, along with squash, pumpkins, melons and gourds. It is widely grown in various parts of the world, favouring warmer climate. In the tropics, it is grown outdoors all year round while in higher latitude regions, it is commonly grown in greenhouse. The immature cucumber fruits are commonly eaten fresh in salads, culinary cooking and pickling. According to FAO (2006), cucumber is the fourth most widely produced vegetable.

The cucumber we know is not found in the wild. However, it is believed to originate from the Himalayas, where a closely related *Cucumis hardwickii* still grows. There are many varieties of cucumber, differing in size, shape, spine and colour. The following subtopics discuss the different types of cucumber, its optimal growing conditions, development stages and farming practices.

Types of Cucumber

Cucumber cultivars may be classified as pickling, slicing, greenhouse or gherkin. Slicing cucumbers are long and tapered with smooth green skin with white spines. This type is commonly eaten raw or served in fresh salads. Pickling cucumbers tend to be blunt, angular, warty and light green with black or white spines. As its name suggests, it is commonly pickled with vinegar. Greenhouse cucumber is a type of seedless slicing cucumber which does not need pollination to bear fruit. It is long and thin with dark green skin. Gherkins (*Cucumis anguria*) are a different species from the common cucumber. It is small, oval and prickly. Gherkins are primarily pickled whole. Cucumbers are normally green, but there are also other white or yellow coloured cucumber types. Most cucumbers mature to become orange, yellow or white in colour. The general types of cucumber are shown in Figure 2.2.



Figure 2.2: The general types of cucumber.

Optimal Growing Conditions

Cucumbers grow best in a warm climate with average day temperature of 30°C and optimum night temperature about 18-21°C. Temperatures above 32°C or below 15°C will slow growth and cause bitterness in the fruit. Cucumber is a shallow rooted crop which prefers loose soil with plenty of organic matter and pH between 6.5 and 7.5. It needs frequent watering but the soil must be well drained to prevent water-logging. Cucumber plants require low nitrogen, high potassium and high phosphorous content for good fruit development.

Development Stages

The growth of a cucumber plant is exponential – slow in the beginning and rapid development as it approaches harvesting stage. Seed germination occurs within 3-7 days of planting at optimal temperature of 20–25°C. By the third week after sowing, the seedlings would have developed the first true leaf which resembles the triangular shape of cucumber leaves. This is when the cucumber seedlings can be transplanted. Flowering stage begins at about 6 weeks after sowing. On a normal cucumber plant, the first flowers that appear are male which will drop after blooming and will not bear fruit. Depending on genetics, day length and temperature, subsequent flowers may be both male and female. Harvest typically begins 12 days after pollination. Immature cucumbers are harvested when the fruit have reached a desired size. Once the harvest stage has begun, cucumber fruits can be harvested once every few days. Figure 2.4 shows the development stages of cucumber from sowing, germination stage, rosette forming stage, head formation stage and harvesting stage.



Figure 2.3: Development stages of cucumber.

Farming Practices

Irrigation is the process of supplying water to the soil using artificial methods. For cucumber, two types of irrigation are commonly used. First type of irrigation is through the use of sprinklers which sprinkle fine drops of water onto the cucumber and soil from overhead. This is usually used after the transplanting stage, when the roots have insufficient contact with the soil to take in enough water. So, wetting the cucumber leaves cools, humidifies and minimized the risk of plant stress. The second form of irrigation is drip irrigation, which provides a constant water supply to the base of the plant. This method reduces evaporation, runoff and the transport of pathogen inoculums from the soil to the leaves.

Fertilizers are a mixture of chemicals that are applied to promote the growth of the plant or its fruit. There are two types of fertilizers which are organic fertilizers and inorganic fertilizers. Organic fertilizers consist of decayed plant or animal matter while inorganic fertilizers consist of chemicals and minerals such as nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, boron, copper, molybdenum, nickel, chlorine, and zinc. Although there are many chemical elements in the fertilizer, the primary ingredients are nitrogen, phosphorus and potassium, thus giving it the name N-P-K fertilizer.

Fertigation is the concept of applying soluble fertilizers through the irrigation system to the plant. This is method is gaining popularity in the agriculture industry because it enables precise application of fertilizers and reduces the amount of fertilizers used as compared to dry application. The irrigation method most commonly used in fertigation is drip irrigation.

Pesticides are mixture of chemical substances that are used to kill or repel pests from consuming or destroying the plant. There are three types of pesticides that are used in cucumber farming which are fungicides, herbicides and insecticides. Fungicides are used to kill or inhibit fungus growth. The most common active ingredient used in fungicides is sulfur. On the other hand, herbicides are used to kill weeds while insecticides are used against insects.

Ralstonia Solanacearum

There are various microscopic and sub-microscopic pathogens that damage or reduce the quality of crops. The three major groups of plant pathogens are fungi, bacteria and viruses. *Ralstonia Solanacearum* is a bacterial pathogen which attacks a wide range of plant species and is found worldwide, favouring warmer and more humid regions. The global distribution of *ralstonia solanacearum* is illustrated in Figure 2.4.



Figure 2.4: Global distribution of ralstonia solanacearum (source: Stansbury, 2001).

There are several symptoms of *ralstonia solanacearum*. The first visible symptom is the wilting of leaves at the ends of branches during the heat of day. This symptom can be easily confused with water stress. As the disease develops, the stem may have a brown discolouration as shown in Figure 2.5 (a). When stems are cut and placed in water, a white slimy mass of bacteria oozes out as shown in Figure 2.5 (b). Eventually, the plant may look stunted and chlorotic before finally dying.



Figure 2.5: Symptoms of *ralstonia solanacearum*. (a) Streaky brown dicolouration in tomato stem. (b) White bacterial ooze from cut tomato stem.

Ralstonia solanacearum can be transmitted through contaminated soil, water, equipment and handlers. The soil can become contaminated with the bacterium through contact with infected plant roots, thus contaminating runoff water. The bacteria can remain in the soil for more than a year. Infections through contaminated equipment or handlers occur when taking clippings from an infected plant and using the same tool on a non-infected plant. The bacterium does not spread aerially. Another possible but less common mode of infection is through infected seeds.

4. FINDINGS

Introduction

This chapter discusses the results obtained in this study to determine the pre-symptom stage of pathogen infection in cucumber and to determine nutrient stress, water stress and light stress can induce a measurable change in electromagnetic spectral response of cucumber during the pre-symptom stage.

Pre-symptom Stage of Pathogen Infection

The pre-symptom stage of pathogen infection is the duration of time in which the plant has been infected but shows no apparent symptoms.





The percent difference method was used to prove that the data was collected during the pre-symptom stage of *ralstonia solanacearum* in cucumber plant. The mean percent difference in reflectance of inoculated and non-inoculated cucumber leaf is shown in Figure 4.1. The mean percent difference throughout the duration of data collection is less than 15%. This means that the average difference in reflectance values of inoculated and non-inoculated cucumber leaves in normal condition is very small and can be ignored. Therefore, it can be concluded that the data has been collected during the pre-symptom stage.

Vegetation Index

The spectral measurements of cucumber in normal condition, nutrient stress, water stress and light stress were quantitatively compared using a combination of vegetation indices such as the Normalized Difference Vegetation Index (NDVI), Nitrogen Reflectance Index (NRI), Structure Insensitive Pigment Index (SIPI), Triangular Vegetation Index (TVI) and Modified Chlorophyll Absorption Ratio Index (MCARI). Using the percent difference of vegetation index values, the difference in spectral signature was quantified.





The plot of NDVI values for cucumber in nutrient stress in Figure 4.2 shows that there is a slight decrease from day 1 to day 10. However, the NDVI values for inoculated and non inoculated cucumber in nutrient stress and normal condition is very similar throughout the 10 days after inoculation.



Figure 4.3: Daily plot of NRI values for cucumber leaves in normal condition and nutrient stress.

The plot of NRI values for cucumber in nutrient stress in Figure 4.3 shows that the non-inoculated cucumber in nutrient stress is slightly higher compared to the inoculated cucumber in nutrient stress on day 1 and 4. This indicates that non-inoculated cucumber in nutrient stress had initially slightly higher chlorophyll absorption. However, the NRI values for inoculated and non inoculated cucumber in nutrient stress are the same from day 6 to day 10.



Figure 4.4: Daily plot of SIPI values for cucumber leaves in normal condition and nutrient stress.

The plot of SIPI values for cucumber in nutrient stress and normal condition in Figure 4.4 shows that the SIPI values for cucumber in nutrient stress and normal condition is the same from day 1 to day 10. This means that both the ratio of carotene and chlorophyll absorption is similar for the cucumber plants in both conditions.



Figure 4.5: Daily plot of TVI values for cucumber leaves in normal condition and nutrient stress.

In the plot of TVI values for cucumber in normal condition and nutrient stress (Figure 4.5), the TVI values for non-inoculated cucumber in nutrient stress is slightly higher on day 1 to day 6. However, the TVI values for inoculated and non inoculated cucumber in nutrient stress are similar from day 9 to day 10.



Figure 4.6: Daily plot of MCARI values for cucumber leaves in normal condition and nutrient stress.

The plot of MCARI values for cucumber in nutrient stress in Figure 4.6 shows that the cucumber in nutrient stress was higher than cucumber in normal condition from day 1 to day 9 with the non-inoculated cucumber in nutrient stress being higher than the inoculated cucumber on day 1 to day 4. This condition was due to higher photosynthetic activity in cucumber in nutrient stress in the initial stage and became similar to cucumber in normal condition on day 10.

From the five graphs comparing vegetation index values for cucumber in normal condition and cucumber in nutrient stress, there was initially higher chlorophyll absorption for cucumber in nutrient stress compared to cucumber in normal condition. However, the spectral response for cucumber in nutrient stress and normal condition became similar on day 9 and day 10. Therefore, nutrient stress was not able to induce a measurable change in spectral response in inoculated cucumber.



Cucumber in Water Stress

Figure 4.7: Daily plot of NDVI values for cucumber leaves in normal condition and water stress.

The plot of NDVI values for cucumber in normal condition and water stress in Figure 4.7 shows that the NDVI values for cucumber in water stress decreased on day 9 to day 10. This indicates a decrease in chlorophyll absorption in both inoculated and non-inoculated cucumber in water stress.



Figure 4.8: Daily plot of NRI values for cucumber leaves in normal condition and water stress.

The plot of NRI values for cucumber in normal condition and water stress in Figure 4.8 shows that the NRI values for cucumber in water stress is similar to cucumber in normal condition.



Figure 4.9: Daily plot of SIPI values for cucumber leaves in normal condition and water stress.

The plot of SIPI values for cucumber in normal condition and water stress in Figure 4.9 shows that the SIPI values for cucumber in water stress increased on day 9 to day 10. This indicates lower chlorophyll absorption than carotene absorption in cucumber in water stress.



Figure 4.10: Daily plot of TVI values for cucumber leaves in normal condition and water stress.

In Figure 4.10, the plot of TVI values for cucumber in normal condition and water stress shows a decrease from day 9 and day 10. This indicates a decrease in chlorophyll absorption in both inoculated and non-inoculated cucumber in water stress.





The plot of MCARI values for cucumber in normal condition and water stress in Figure 4.11 shows that the MCARI value for cucumber in water stress is considerably higher than that of cucumber in normal condition. The MCARI value for inoculated cucumber in water stress is higher than non-inoculated cucumber in water stress on day 4 and day 6 because there was a high increase in green reflectance.

From the NDVI, NRI, SIPI and TVI plots for cucumber in water stress, there was a decrease in photosynthetic activity in the cucumber plants in water stress. However, there was no difference between the vegetation index values between the inoculated cucumber and non-inoculated cucumber in water stress. Therefore, the change in spectral response was due to water stress and water stress was not able to induce a measurable change in spectral response in inoculated cucumber.



Figure 4.12: Daily plot of NDVI values for cucumber leaves in normal condition and light stress.

The plot of NDVI values for cucumber in light stress in Figure 4.12 shows that there is a slight decrease from day 1 to day 10. However, the NDVI values for inoculated and non inoculated cucumber in light stress and normal condition is very similar throughout the 10 days after inoculation.



Figure 4.13: Daily plot of NRI values for cucumber leaves in normal condition and light stress.

From the plot of NRI values for cucumber in normal condition and light stress in Figure 4.13, the inoculated cucumber in light stress is noticeably lower than the non-inoculated cucumber in light stress and the cucumber in normal condition from day 6 to day 10. This is due to lower chlorophyll absorption in the inoculated cucumber in light stress.



Figure 4.14: Daily plot of SIPI values for cucumber leaves in normal condition and light stress.

The plot of SIPI values for cucumber in light stress and normal condition in Figure 4.14 shows that the SIPI values for cucumber in light stress and normal condition is the same from day 1 to day 10. This means that both the ratio of carotene and chlorophyll absorption is similar for the cucumber plants in both conditions.





From the plot of TVI values for cucumber in normal condition and light stress in Figure 4.15, the values for inoculated cucumber in light stress is slightly lower compared to non-inoculated cucumber in light stress and the cucumber in normal condition. This is due to lower chlorophyll absorption in inoculated cucumber in light stress.



Figure 4.16: Daily plot of MCARI values for cucumber leaves in normal condition and light stress.

In Figure 4.16, the MCARI values for inoculated cucumber in light stress are considerably lower than that of non-inoculated cucumber in light stress and cucumber in normal condition from day 6 to day 10. This indicates that there is lower chlorophyll absorption in inoculated cucumber in light stress.

While the NDVI and SIPI plots for cucumber in normal condition and light stress show no difference, the NRI, TVI and MCARI plots show that there was a decrease in chlorophyll absorption in the inoculated cucumber in light stress compared to the non-inoculated cucumber in light stress and cucumber in normal condition. This indicates that light stress has induced a condition of less chlorophyll absorption in the inoculated cucumber.



Percent Difference of Vegetation Index

Figure 4.17: Percent difference of vegetation index values between inoculated and noninoculated cucumber in nutrient stress.

Figure 4.17 shows the percent difference of vegetation index values for inoculated and non-inoculated cucumber in nutrient stress. The MCARI and TVI values show temporary difference in day 4 and day 6. The difference is only temporary and thus cannot be considered as an induced change in spectral response. The other vegetation indices show no detectable difference between inoculated and non-inoculated cucumber in nutrient stress.



Figure 4.18: Percent difference of vegetation index values between inoculated and noninoculated cucumber in water stress.

Figure 4.18 shows the percent difference of vegetation index values for inoculated and non-inoculated cucumber in water stress. The MCARI was able to detect a difference between inoculated and non-inoculated cucumber in water stress on day 4 and day 6. This difference is a result of reduced chlorophyll absorption in inoculated cucumber due to less water content compared to non-inoculated cucumber in water stress.



Figure 4.19: Percent difference of vegetation index values between inoculated and noninoculated cucumber in light stress.

Figure 4.19 shows the percent difference of vegetation index values for inoculated and non-inoculated cucumber in light stress. The TVI shows a difference between inoculated and non-inoculated cucumber on day 6 and day 9 but there is no difference on day 10. The NRI and MCARI show a difference between inoculated and non-inoculated cucumber from day 4 onwards. This shows that light stress can induce a change in spectral response in inoculated cucumber during the pre-symptom stage.

Visual Comparison

Figure 4.20 shows samples of both inoculated and non-inoculated cucumber leaves from each set conditions that were taken on day 10. The inoculated and non-inoculated cucumber leaves in normal condition looks similar. This is consistent with the findings in Section 4.2 where the mean percent difference between inoculated and non-inoculated cucumber in normal condition is less than 15%, proving that the pre-symptom stage exists during the 10 days of data collection.

The cucumber leaves in nutrient stress show little difference between the inoculated and non-inoculated cucumber. The cucumber leaves in water stress show symptoms of water stress but show little difference between inoculated and non-inoculated cucumber in water stress. On the other hand, the inoculated cucumber in light stress is noticeably darker compared to the non-inoculated cucumber in light stress. All these observations are consistent and in support of the findings of the previous sections of this chapter.



Figure 4.20: (a) Non-inoculated cucumber in normal condition. (b) Inoculated cucumber in normal condition. (c) Non-inoculated cucumber in nutrient stress. (d) Inoculated cucumber in nutrient stress. (e) Non-inoculated cucumber in water stress. (f) Inoculated cucumber in water stress. (g) Non-inoculated cucumber in light stress. (h) Inoculated cucumber in light stress.

5. CONCLUSION

The objectives of this study is to prove the occurrence of pre-symptom stage of the infection of ralstonia solanacearum in cucumber plants and to determine the possibility of inducing a measurable spectral change by using nutrient, light exposure and water stress during the pre-symptom stage. The result of this study is concluded and recommendations for future research are given in this chapter.

Conclusion

The pre-symptom stage is the period where the plant is infected but symptoms have not yet developed. The difference in reflectance of inoculated and non-inoculated cucumber is less than 15% during the 10 days of data collection. Thus, it is proven that the presymptom stage exists at least 10 days after inoculation. This is in fulfillment of the first objective of this study.

From the vegetation indices used, it can be concluded that there is little or no difference between the spectral response of the inoculated and non-inoculated cucumber in nutrient stress and the cucumber in normal condition. The cucumber in water stress showed symptoms of water stress and showed no difference between the spectral responses of inoculated and non-inoculated cucumber in water stress. On the other hand, the cucumber in light stress showed a difference in spectral responses of inoculated and non-inoculated cucumber in light stress. This suggests that light stress is able to induce a change in spectral response during the pre-symptom stage.

The percent difference analysis of vegetation index values have proven that a change in spectral reflectance was successfully induced during the pre-symptom stage of *ralstonia solanacearum* infection of cucumber using light stress as an inducing factor. This phenomenon was detected using NRI, TVI and MCARI.

From this study, it is possible to detect crop diseases in the early stage of infection by inducing a measurable change in spectral response by applying light stress. Through the exploration and expansion of this study, its eventual application in agricultural precision farming can significantly prevent unnecessary loss of productivity due to crop diseases.

Recommendation

This study has merely proven the hypothesis that a measurable change in spectral response can be induced during the pre-symptom stage of *ralstonia solanacearum* infection of cucumber. This serves as a stepping stone for countless research towards the implementation of this technique in precision farming practices. Recommendations for future research include:

a. Using other combinations of crop, pathogen and inducing factors to improve the applicability of the results of this study. The result of this study may or may not apply to other combinations of crops, pathogens and inducing factors. There was insufficient time and resources to test the hypothesis of this study using other types of crops, pathogens and inducing factors.

b. Higher duplication of samples to reduce random errors that may have an effect on the overall result. Although the plants were grown in a controlled environment, there may be differences among plants that cannot be avoided such as relative distance from light source. Therefore, more plant samples should be grown and more spectroradiometer measurements should be taken to reduce the effects of random errors.

c. Incorporation of remotely sensed imagery such as hyperspectral imagery to enable the application of the result of this study in precision farming practices. The intention of conducting this study is to ultimately apply the results of this study to detect crop diseases in the early stages of infection to decrease losses of productivity. Therefore, the results of this study should be tested using remotely sensed imagery of crop fields.

6. RESEARCH OUTPUT

- 6.1 Citation Details of Articles NIL
- 6.2 Citation Details of Conference Papers NIL
- 6.3 Citation Details of Other Publications books / standards etc. NIL
- 6.4 Details of IPR NIL

7. HUMAN CAPITAL DEVELOPMENT

7.1 Details of Human Capital Development (Name and qualification sought)- NIL

8. AWARDS / ACHIEVEMENT

8.1 Details of Recognition Received - NIL

9. **REFERENCES**

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10. APPENDIXES