AN INVESTIGATION INTO THE DETECTION OF SUGARCANE AFRICAN STALK BORER (*ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)) USING HYPERSPECTRAL DATA (SPECTRORADIOMETRY)

BY

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ABSTRACT

The South African Sugarcane production is one of the world's leading sugarcane (*Saccharum* spp. Hybrid) producers. However, in recent years *Eldana saccharina* Walker has been the most destructive pest in South African sugarcane production, causing great crop loses per annum and is the most important factor limiting sugarcane productivity. The pest has been monitored using a traditional visual approach whereby a representative sample of stalks is taken from a field and split longitudinally to assess damage and count the number of *E. saccharina* larvae and pupae. However, this approach is time-consuming, labour intensive and sometimes biased as only easily accessible areas are often surveyed. In order to investigate a more economical but equally effective survey methodology, this study aimed to determine the potential of using hyperspectral remote sensing (spectroradiometry) for identifying sugarcane attacked by *E. saccharina*. A hand-held spectroradiometer *ASD Field Spec® 3* was used to collect leaf spectral measurements of sugarcane plants from a potted-plant trial taking place under shade house conditions at the South African Sugarcane Research Institute (SASRI). In this trial, nitrogen (N) and silicon (Si) fertilizers were applied at known levels to sugarcane varieties. Varieties were either resistant or intermediate resistant or susceptible to *E. saccharina* attack. In addition, watering regimes and artificial infestation of *E. saccharina* were carefully controlled. Results illustrated that severe *E. saccharina* infestation increased spectral reflectance throughout the whole spectrum range (400 – 2500 nm) and caused a red-edge shift to the shorter wavelength. *Eldana saccharina* stalk damage was also linearly related to modified normalized difference vegetation index (mNDVI) using R_{2025} and R_{2200} (R^2 = 0.69). It was concluded that hyperspectral data has a potential for use in monitoring *E. saccharina* in sugarcane rapidly and non-destructively under controlled conditions. A follow-up study is recommended in field conditions and using airborne and/or spaceborne hyperspectral sensors.

PREFACE

The experimental work described in this dissertation was carried out in the School of Environmental Sciences, University of KwaZulu-Natal, Durban, from January 2007 to December 2008, under the supervision of Prof Fethi B. Ahmed.

These studies represent original work by the author and have not been submitted in any form for any degree or diploma to any other tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION – PLAGIARISM

I, Tholang Alfred Mokhele, declare that

1. The research reported in this dissertation, except where otherwise indicated, is my original research.

2. This dissertation has not been submitted for any degree or examination at any other university.

3. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed: ……. Date: ……..

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DEDICATION

This Dissertation is dedicated to my first born daughter, Lemohang Jessica Mokhele.

ABBREVIATIONS

ANOVA - Analysis of Variance
ASCII - American Standard Code for Information Interchange
ASD - Analytical Spectral Devices
AVHRR - Advanced Very High Resolution Radiometer
AVIRIS - Airborne Visible/Infrared Imaging Spectrometer
AWC - Absolute Water Content
BEE - Black Economic Empowerment
Ca - Calcium
CASI - Compact Airborne Spectrographic Imager
CCCI - Canopy Crop Chlorophyll Index
CI - Carter Index
CO_2 - Carbon Dioxide
CWSI - Crop Water Stress Index
D_i - Derivative (1^{st}) at Waveband i
DMSV - Digital Multi-Spectral Video
EnMAP - Environmental Mapping and Analysis Program
EO - Earth Observation
ETM+ - Enhanced Thermatic Mapper Plus
EVI - Enhanced Vegetation Index
FR - Full Range
GER - Geophysical Environmental Research
GPS - Global Positioning Systems
HERO - Hyperspectral Environment and Resource Observer
HIRIS - High Resolution Imaging Spectrometer
HYMAP - Hyperspectral Mapper
IEEE - Institute of Electrical and Electronics Engineers
IPM - Integrated Pest Management
IR - Infrared
K - Potassium
LA - Leaf Area
LAI - Leaf Area Index
MERIS - Medium Resolution Imaging Spectrometer

Mg - Magnesium
MIR - Mid Infrared
MISR - Multiangle Imaging Spectroradiometer
MODIS - Moderate Resolution Imaging Spectroradiometer
MLR - Multiple Linear Regressions
MSS - Multispectral Scanners
N - Nitrogen
NDI - Normalized Difference Index
NDRE - Normalized Difference Red Edge Index
NDVI - Normalized Difference Vegetation Index
NIR - Near Infrared
NOAA - National Oceanographic and Atmospheric Administration
NPCI - Normalized Pigment Chlorophyll Index
NRI - Nitrogen Reflectance Index
NY - New York
O_2 - Oxygen
P - Phosphorus
P_n - Photosynthetic Rate
PRI - Photochemical Reflectance Index
PVI - Perpendicular Vegetation Index
R - Rand
REI - Red Edge Index
REP - Red-Edge Position
R_i - Reflectance at Waveband *i*
RSA - Republic of South Africa
RV - Recoverable Value
RVI - Ratio Vegetation Index
SASRI - South African Sugarcane Research Institute
SAVI - Soil-adjusted Vegetation Index,
SE - Standard Error
Si - Silicon
Sig - Significance
SPOT - Systeme Pour l'Observation de la Terre
SPSS - Statistical Package for Social Sciences

SWIR - Short-wave Infrared
TIR - Thermal Infrared
TM - Thematic Mapper
TVI - Transformed Vegetation Index
UK - United Kingdom
USA - United States of America
UV - Ultraviolet
VI - Vegetation Index
VNIR - Visible Near-Infrared
WBI - Water Band Index
WBR - Water Band Ratio

CHAPTER ONE: INTRODUCTION

1.1 Background

Sugarcane (*Saccharum spp*. Hybrids) is a tall-growing perennial crop grown in tropical and subtropical regions (Muchovej *et al.*, 2005; Abdel-Rahman and Ahmed, 2008). Sugarcane crop is an extremely water intensive and yet an important cash crop. It is an important component of the economy in most countries and apart from being the major source of world's sugar used in human diet, several by-products have been produced from its milling. Recently, there has been an increased interest in biofuel production from its by-products (Masoood and Javed, 2004; Inman-Bamber and Smith, 2005; Inman-Bamber *et al.*, 2005; Abdel-Rahman and Ahmed, 2008).

South African sugarcane production comprises the agricultural activities of sugarcane cultivation with industrial factory production of raw and refined sugar, specialized sugars as well as syrup, including a range of by-products (SASRI, 2007). The South African sugarcane production is known for its major contribution to the national economic growth through sugar production, foreign exchange earnings, job provision, social and sustainable development as well as Black Economic Empowerment (BEE) (Anon, 2007). For example, its annual production is approximately 2,5 million tons of which 50% is exported to other African countries and other continents including North America. Through these export markets, the production generates an average income of R6 billion a year and also contributes approximately R2 billion to the country's foreign exchange earnings (SASRI, 2007).

However, there are major factors which limit the productivity of sugarcane in South Africa. These include drought especially in rainfed areas, high climatic variability, poor soils as well as pests *(E. saccharina)* and diseases. The subtropical conditions in the South African sugarcane production areas also limit the growth potential of South African sugarcane as it is essentially a tropical crop (Coetzee, 2003). Some of these factors influence others, for example, lack of rainfall and soils susceptible to moisture stress cause moisture stressed sugarcane, which is very susceptible to attack and population build up of pests such as *E. saccharina* (Atkinson *et al.,* 1981; Atkinson and Nuss, 1989; SASRI, 2005). Among these

factors, *E. saccharina* (an insect indigenous to Africa) is a major pest in South Africa and the most important factor limiting sugarcane productivity, causing great crop losses per annum (Redshaw and Donaldson, 2002; Meyer and Keeping, 2005a).

The detection of *E. saccharina* in South African sugarcane is very important. It is worth highlighting to indicate factors influencing this pest in South African sugarcane. These include plant nitrogen (N) and silicon (Si), sugarcane age, water stress and resistance or susceptibility of sugarcane varieties. Meyer and Keeping (2005a) indicated that N and Si play important roles in the susceptibility and resistance of a range of crops to stalk borer *(E. saccharina)* damage. For instance, high Si contents interfere with the feeding of *E. saccharina* larvae by damaging their mandibles (Savant *et al.,* 1999; Kvedaras *et al.,* 2007; Kvedaras and Keeping, 2007). High N levels on the other hand are linked with high *E. saccharina* infestations because of shortened development time of the insect (Atkinson and Nuss, 1989; SASRI, 2005). However, plant stress causes a redistribution of N from the leaves and growing shoots to the stalk where *E. saccharina* larvae feed (Atkinson and Nuss, 1989), therefore there might be a reduction in foliar N concentration. In addition, recent studies show that N/Si ratio is correlated with *E. saccharina* damage and hence sugarcane with foliar N/Si ratio greater than 2 is associated with increasing risk of *E. saccharina* borer damage (Meyer and Keeping, 2005a; SASRI, 2005).

Sugarcane age is another factor influencing *E. saccharina* incidence as there is a strong positive correlation between cane age and *E. saccharina* larval population as well as between cane age and *E. saccharina* damage (Atkinson and Nuss, 1989; SASRI, 2005; Goebel and Way, 2007; Way and Goebel, 2007). This is further confirmed by Atachi *et al.* (2005) that post-tasseling stages are more attractive to *E. saccharina* than pre-tasseling ones for all host plants. However, severely stressed sugarcane plants might be infested very early, at 2 – 3 months age due to the presence of dead leaf material (Atkinson and Nuss, 1989).

Water stress is one of the factors influencing *E. saccharina*. Lack of rainfall causes moisture stressed sugarcane, which is very susceptible to attack and population build up of *E. saccharina* (Atkinson and Nuss, 1989; Atkinson *et al.,,* 1989). Atkinson and Nuss (1989) further showed that the infestations of *E. saccharina* are worse in water stressed plants in South Africa. Female moths lay eggs on dry or dead leaves of the host plant, stressed cane

(Atkinson, 1979; SASRI, 2006). These dead and dry leaves can be detected by remote sensing.

Resistance or susceptibility of sugarcane varieties to infestation by *E. saccharina* have been reported (Webster *et al.,* 2005), with varieties ranging from being resistant, intermediately-susceptible and susceptible to infestation by *E. saccharina*. Therefore it is normally recommended that resistant varieties be planted in problem areas such as sandy soils as well as near natural host plants such as sedges (Webster *et al.,* 2005).

1.2 Assessment, Monitoring and Detection of *E. saccharina*

Assessment, monitoring and detection of *E. saccharina* in sugarcane are very important for management decisions as well as prompt decision making. This has been done through traditional or visual approach (Way and Goebel, 2007). The approach involves destructive sampling of cane stalks from the field and then longitudinally splitting them for assessing stalk damage as well as internodes damage by *E. saccharina* and for counting the number of *E. saccharina* larvae and pupae found in the stalks. However, this approach is inefficient as it is time-consuming, labour intensive and sometimes biased as only easily accessible areas are surveyed (Apan *et al.*, 2005). According to Apan *et al.* (2005), remotely sensed data, especially hyperspectral data, can be used to supplement traditional or visual approaches for assessment, monitoring and detection of disease and pest symptoms, and such techniques have advantages over traditional approaches as they can be used to repeatedly collect sample measurements both non-destructively and non-invasively.

Remote sensing techniques have been used as potentially important tools for the identification of nutrient content, chlorophyll content, detection of pests and diseases, water stress, mapping, precision farming, hail damage, crop inventory as well as yield estimation in agricultural crops, for example (Schmidt *et al*., 2001; Kumar *et al*., 2003; Gers, 2004; Datt *et al.*, 2006). Most of the above studies use hyperspectral data. Hyperspectral remote sensing make use of the simultaneous acquisition of data in many relatively narrow and contiguous spectral bands throughout the ultraviolet, visible and infrared portions of the electromagnetic spectrum (Jensen, 2005). However, hyperspectral remote sensing has been used interchangeably with the following terms: imaging spectroscopy, imaging spectrometry and imaging spectroradiometry (van der Meer and de Jong, 2003).

Remote sensing of plants records the plant leaves' spectral responses as influenced by both abiotic and biotic factors. For instance, pests and diseases can induce differences in spectral responses of plant leaves as they (pests and diseases) change the physiological responses to nutrient and environmental stress, biochemistry, and biophysical properties of leaves (Apan *et al.*, 2005). Reflectance-based remote sensing techniques for pest identification capitalize on the fact that most pests affect the outwards appearance of a plant in a particular manner either within the visible or outside the visible spectrum (Abdullah and Umer, undated). However, *E. saccharina* effects on sugarcane leaf physiology are not known. This study proposes to test whether there are physiological changes in sugarcane leaves induced by *E. saccharina*, which result in spectral reflectance differences that can be detected by spectroradiometry.

1.3 Motivation for the Study

Very few remote sensing studies have been undertaken in South Africa on sugarcane agriculture using multispectral sensors (broadband sensors) except recent works by Abdel-Rahman *et al.* (2008a; b) where hyperspectral data were used. Multispectral sensors have fewer channels but these have broadband (~100 μm) which make them average the reflectance over a wide range of wavelength. Due to this averaging characteristic, much data about narrow spectral features are lost or masked by stronger features surrounding those (Kumar *et al*., 2003). Presumably, there are changes in narrow spectral absorption features of sugarcane leaves induced by *E. saccharina* which may go undetected by these multispectral sensors. In contrast, hyperspectral sensors with over 100 contiguous and narrow sensitive bands (~10 nm) can detect these changes in narrow absorption features (Lillesand *et al.,* 2004).

This high sensitivity of hyperspectral data makes it more sensitive and capable in determining reflectance changes induced by *E. saccharina* on sugarcane leaves at leaf-level using spectroradiometry. The results from field or leaf-level application may lead to the use of air- or space-borne hyperspectral imaging, in assessment and detection of *E. saccharina* in sugarcane.

1.4 Aim and Objectives

The aim of this study was to determine the potential use of hyperspectral data (spectroradiometry) for identifying sugarcane plants that are attacked by *E. saccharina*.

The specific objectives of the study were:

(a) To determine if leaf-level spectral reflectance of sugarcane can be used to detect infestation by *E. saccharina*,

(b) To determine if leaf-level spectral reflectance of different sugarcane varieties can be used to detect various levels of *E. saccharina* damage and water stress,

(c) To determine the best hyperspectral narrow-wave bands for sugarcane *E. saccharina* detection and N/Si ratio estimation,

(d) To estimate leaf biochemical concentrations of N and Si in relation to *E. saccharina* incidence and water stress levels.

1.5 Dissertation Outline

Chapter one describes background of sugarcane crop and its production in South Africa. This chapter further highlights the motivation and the aim of the study. Chapter two presents a brief biology, history and economic impact of *E. saccharina.* It further provides a brief description of remote sensing and its theory in agriculture, with some detailed information on general leaf spectral optical properties. This chapter also presents the application of hyperspectral remote sensing in agriculture. Chapter three describes the materials and methods used in this study. In chapter four, the results and findings of this study are presented and discussed. Finally, chapter five presents the conclusions of the study as well as the recommendations made.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter presents the biology, history, distribution and economic importance of *E. saccharina*. It further provides an insight of remote sensing in agriculture, with some detailed description on general leaf spectral properties, with some focus on sugarcane leaf spectral properties. The chapter also discusses the applications of hyperspectral data in agriculture, presenting both imaging spectroradiometry and non-imaging spectroradiometry applications.

2.2 Biology, History, Distribution and Economic Impact of *E. saccharina*

2.2.1 Biology of *E. saccharina*

Eldana saccharina is an insect that is indigenous to Africa. It is a very active and tough insect with yellow-brown colour and rather leathery larval borer which wriggles vigorously when disturbed (Carnegie, 1974; SASRI, 2006). Its larva bores into lower cane stalk where most sucrose is stored. Its presence is known by the frass which it pushes out of the host plant stalk through the holes that it has already bored. It may also change the cane stalk into red colour. At low infestations these holes can be the only indication of *E. saccharina'*s presence while in severe infestations, the entire crop can be destroyed (Carnegie, 1974; SASRI, 2006).

The life cycle of *E. saccharina* consists of four stages which include, egg, larva, pupa and moth stages. The duration of the life cycle is extremely variable as it depends on many factors such as the quality of food supply and the ambient temperature (Atkinson and Carnegie, 1989). For example, the development of eggs takes 12 days under a mean temperature of 17 °C and 5 days under mean temperature of 26 °C (Croix, 1992). This means that the hotter the weather, the shorter the life cycle will be, but all in all the life cycle lasts for 1 – 2 months (SASRI, 2005).

The female moth, which is light brown in colour, is estimated to live for about one week. It flies looking for a mate and lays most of its eggs within 2 – 3 days of mating. Eggs are laid on dry or dead leaves of the host plant (Atkinson, 1979; SASRI, 2006). These dead and dry leaves can be detected by remote sensing. Croix (1992) stated that each female moth lays

about 100 – 200 eggs. The laid eggs take about a week and then the minute first stage larvae (hatching larvae) emerge. The eggs are white, but if fertile they turn pink and finally become brownish or blackish on the day before hatching. Infertile eggs normally turn yellow instead of pink and shrivel. Generally, the largest specimens produce female moths while the smallest yield male (Dick, 1945). The first stage larvae (hatching larvae) feed as scavengers on the exterior side of the host plant stalk (Carnegie, 1974; SASRI, 2006), and on the decaying leaf matter on the leaf surface (Croix, 1992). Then bore into the stalk where they spend the entire larval period (Dick, 1945; Carnegie, 1974; SASRI, 2006). The duration of the larvae is approximately 50 days. The mature larva, the most damaging stage as they feed on the internal soft tissue of the plant stalk, spins a protective cocoon and pupates within it (Carnegie, 1974).

The pupa would then harden and change from yellow-brown colour to red-brown colour a day after its emergence. Atkinson and Carnegie (1989) stated that at the prevailing temperatures in spring the duration of the pupal stage would be about 10 days, in order for the onset of pupation to be soon followed by eclosion, which is the emergence of an insect from its pupal case or egg. Then the moths (adults) will start emerging from the pupae at sunset till about 9 pm. Thus, during the day, the moths are inactive hence mating, oviposition and locomotion take place between sunset and sunsrise (Dick, 1945). Even though the breeding process is continuous, SASRI (2006) showed that there are two moth peaks around April and November. Conlong and Kasl (2000) stated that all life stages of *E. saccharina* are very cryptic which makes it difficult to control this insect by conventional pest management strategies.

2.2.2 History of *E. saccharina*

The first record on *E. saccharina* in Africa was in Siera Leone, in 1865 (Dick, 1945; Carnegie, 1974; Conlong, 1994; Horton *et al*., 2002). It was found attacking maize, sugarcane and sorghum. During the early 1900s, the *E. saccharina* moth was found in Tanzania and Beira in Mozambique (Dick, 1945; Carnegie, 1974; Atkinson *et al.,* 1981; Croix, 1992). Croix (1992) also mentioned that in 1925 *E. saccharina* was found attacking maize in French West Africa and in 1928 the insect was found in South Africa, at the Nyalazi river near Mtubatuba, though the infestations were very low.

In 1939, the first outbreak of *E. saccharina* took place, it was when this pest became known as a pest of importance in South African sugarcane restricted to Umfolozi River Flats in the northern KwaZulu-Natal (Atkinson, 1979; Atkinson *et al.,* 1981; Croix, 1992; SASRI, 2006). However this outbreak was not for long for unknown reasons (Coetzee, 2003). In 1944, Girling 1972 in Croix (1992) reported that *E. saccharina* was found on cassava in Zaire though he was a little confused about its identification.

In the 1970s, the second outbreak was discovered in South Africa, this time it was permanent and ongoing spreading from Umfolozi area to the rest of the coastal areas where sugarcane was grown, this was as far as Malelane and Port Shepstone as well as Hluhluwe extending even into Swaziland (Carnegie, 1974; Atkinson *et al.,* 1981; Croix, 1992; Coetzee, 2003; SASRI, 2006). In 1977, the spread of *E. saccharina* infestations further got into the south coastal areas on KwaZulu-Natal as far as Port Shepstone (Atkinson *et al.,* 1981; Croix, 1992). In the 1980s, much of the spread of sugarcane *E. saccharina* has been associated with years of low rainfall and extremely dry conditions, especially in 1983 and 1985 (Atkinson and Carnegie, 1989). SASRI (2006) postulated that the most recent area that has been invaded is the highlands of KwaZulu-Natal due to drought in 1992 – 1994, though the incidences were reduced by cold winters of these highlands.

2.2.3 Distribution of *E. saccharina*

Eldana saccharina is widely distributed in Africa (Kfir *et al.,* 2002). In Siera Leone, where the pest was first described, the crop hosts were sugarcane, maize and sorghum (Dick, 1945; Horton *et al*., 2002). Horton *et al*. (2002) further postulated that the shifting of *E. saccharina* from its natural or indigenous hosts to the crop hosts was due to the fact that the crop plants were cultivated in swampy areas, replacing the indigenous sedges and grasses, containing *E. saccharina.* Although there are similarities in terms of its behavior throughout Africa, there are also some differences. In West and East Africa, *E. saccharina* borer mainly infests the upper parts of the stalks while in southern Africa it infests the lower parts of the stalks where most sucrose is stored (Kfir *et al.,* 2002; Coetzee, 2003). In addition, *E. saccharina* is known to attack mainly maize, rice and sugarcane in West Africa while it is a major pest for sugarcane in southern Africa and rarely causes damage to maize (Kfir *et al.,* 2002).

E. saccharina has a tropical and subtropical distribution from sub-saharan West Africa, across East Africa and down the African east coast to coastal KwaZulu-Natal (Atkinson and Carnegie, 1989; Conlong and Kasl, 2000). SASRI (2005) indicated that this insect occurs more commonly in a number of indigenous grasses and sedges *(Cyperus papyrus* and *Cyperus dives)*, especially those found in wetlands and along the coast as well as along river banks and in rain-fed areas. Conlong and Kasl (2000) stated that larvae and pupae feed on the rhizomes in sedges while they feed on stalk in sugarcane and generally on the lower half.

Eldana saccharina has been found in number of crops such as maize, millet, sorghum and rice. However, it has not been regarded as a serious pest as it attacks these crops at older stage, hence does not cause significant loss in yield production (Coetzee, 2003). In some countries such as Malawi and Zimbabwe *E. saccharina* affects natural host plants but sugar cane is not affected (Atkinson and Nuss, 1989; Atkinson and Carnegie, 1989). In Uganda, *E. saccharina* was restricted to wild hosts but later expanded its host range to maize, sorghum and sugarcane plants (Overholt *et al.,* 1996). In Nigeria, *E. saccharina* has been found as a dominant stem borer on millet, sorghum and rice crops (Harries 1962 in Croix, 1992).

In South Africa, where sugarcane is the major host plant as in Swaziland, the distribution of *E. saccharina* is limited by the winter temperature in the centre and south of the cane belt (Atkinson, 1979). Atkinson and Nuss (1989) showed that the infestations of *E. saccharina* are higher in intensively grown sugarcane than in peasant grown sugarcane due to different levels of technologies applied and are worse in water stressed plants in South Africa. “In one particular area of the South African sugarcane belt, the Zululand region, infestation is so serious that it not only causes frequent marked losses in sucrose yields but it has, at times, caused consignments to be rejected at the mill or ratoon failure after harvest. Elsewhere in the sugarcane belt, the pest has in recent years invaded sugarcane further south and at higher altitudes than where it used to be found” (Atkinson and Carnegie,1989: 61).

2.2.4 Economic Negative Impacts of *E. saccharina* on South African Sugarcane Production

In some areas such as Zululand sugarcane belt, *E. saccharina* infestation was so serious that it even resulted in consignments being rejected at the mill or ratoon failures after harvest (Atkinson and Carnegie, 1989). Economic losses are also encountered when growers are forced to harvest cane annually, at its younger age, due to infestations of *E. saccharina* as

more sucrose accumulates preferentially in mature cane. These losses are further increased as there will be more expenses for frequent cultivation, maintenance as well as harvesting if cane cycle is 12 months (Coetzee, 2003).

The economic negative impact of *E. saccharina* on sugarcane is highly significant as it causes losses of about R250 million per annum in South Africa (Horton *et al*., 2002; Hurly and Buchanan, 2006). According to recent studies, it had caused losses of about R153 million in the 2003/2004 milling season, thus approximately 143 000 tonnes Recoverable Value (RV) (Goebel and Way, 2007). It is even estimated that the *E. saccharina* larval feeding causes 0.1 % sucrose loss for every 1 % of sugarcane stalks damaged (Horton *et al.,* 2002). SASRI (2005) postulated that it has been estimated that for every 1 *E. saccharina* per 100 sugarcane stalks, there is a loss of 0.5 ton cane per hectare. This pest has a great impact on sucrose yield as compared to cane weight with a decrease of 21.4 – 20 % in highly infested plots (Goebel and Way, 2007).

In an attempt to reduce these significant economic losses caused by *E. saccharina*, various control methods have been applied, even though control of this pest has proved problematic in South Africa (Horton *et al*., 2002). In the past the main control method has been to harvest earlier if the infestation was becoming severe and the mill would reject cane only if it was severely damaged (Horton *et al*., 2002; Webster *et al.*, 2002). SASRI (2006) stated that there is no single measure that can provide an answer to the *E. saccharina* problem, hence only Integrated Pest Management (IPM), which is a combination of selective control measures that can work at appropriate times in the cane crop or pest cycle. Recent IPM practices in South African sugarcane production are stipulated and discussed in Webster *et al.* (2002). Even though these IPM practices are done, *E. saccharina* pest persists as a major constraint to South African sugarcane production, hence definitive control strategies remain to be developed (Goebel and Way, 2007).

2.3 Remote Sensing

Remote Sensing is defined as the science of obtaining information about an object through the analysis of data acquired by a device that is not in direct contact with the object (Lillesand and Kiefer, 2000; ASD, 2006). The quantity that is mostly measured in day-to-day remote sensing systems is electromagnetic energy emanating from objects of interest (Campbell, 2002). This

electromagnetic energy is commonly recorded according to its wavelength location within the electromagnetic spectrum. The spectrum ranges from Cosmic rays, Gamma rays, X rays, Ultraviolet (UV), Visible, Infrared (IR), Microwave up-to Radio waves (Legg, 1992; Lillesand and Kiefer, 2000).

2.3.1 Hyperspectral and Multispectral Sensors

Multispectral data collect spectral data in a few broad spectral bands, non contiguous ranges of the electromagnetic spectrum, which means a single band represents the average of a relatively large portion of the spectrum. Hyperspectral remote sensors provide spectral data in many relatively narrow and contiguous spectral bands throughout the ultraviolet, visible and infrared portions of the electromagnetic spectrum (Oskin and Roberts, 2004; Jensen, 2005). This study focuses on hyperspectral data. Hyperspectral remote sensing has been used interchangeably with the following terms: imaging spectroscopy, imaging spectrometry and spectroradiometry (van der Meer and de Jong, 2003; Oskin and Roberts, 2004). Imaging spectroscopy is defined as the branch of physics which deals with the production, transmission, measurement and interpretation of electromagnetic spectra while spectrometry or spectroradiometry, which is derived from spectro-photometry, is defined as the measure of photons as a function of wavelength (Kumar *et al*., 2003). The only difference between spectrometry and spectroradiometry is that, in spectroradiometry also spectral measurements of radiance and irradiance are available (ASD, 2006).

Hyperspectral data have some strengths and limitations over multispectral data in agricultural applications. In relation to strengths, for instance, hyperspectral sensors with 200 or more contiguous and narrow sensitive bands can detect changes in narrow absorption features that are lost within the relatively coarse bandwidths of various bands of multispectral sensors. Multispectral sensors average the reflectance over a wide range and hence narrow spectral features are lost or masked by other stronger features surrounding them (Kumar *et al*., 2003; Lillesand *et al.,* 2004; Govender *et al.,* 2007). “For this reason hyperspectral remote sensing is a strong alternative for significant advancement in the understanding of the earth and environment” (Kumar *et al*., 2003: 111 – 112).

However, the main limitation encountered in hyperspectral remote sensing is that, hyperspectral data contain large amounts of redundant information for any given application.

This will require determining the optimum number of wavebands, waveband centres, and waveband widths required to maximize information. The effort should lead to identifying wavebands that are most critical to a particular application, and in eliminating the need to gather and transmit data from a huge number of hyperspectral wavebands by designing a sensor with optimum number of wavebands (Thenkabail *et al.,* 2004). Another limitation of hyperspectral data is that, data acquired by very fine spatial resolution sensors are used for localized small study areas in contrary to multispectral with their coarser bands which cover a larger study areas (Lucas *et al.,* 2008).

2.4 Remote Sensing in Agriculture

2.4.1 Brief Overview

The use of remote sensing in agriculture is one of the main application fields of remote sensing techniques (Clevers, 1999; Clevers and Jongschaap, 2001). Remote sensing technology can be used to provide both quantitative and timely information on agricultural crops during their growing season (Yang *et al*., 2008). Remote sensing techniques have been used as potentially important tools for the estimation of nutrient content, chlorophyll content, detection of pests and diseases, water stress, mapping, precision farming, hail damage, crop inventory as well as yield estimation in agricultural crops over wide areas with the ability to evaluate information in an unbiased way (Schmidt *et al*., 2001; Kumar *et al*., 2003; Gers, 2004; Datt *et al.*, 2006).

2.4.2 Spectral Signatures, Spectral Reflectance and Leaf Optical Properties

When light interacts with any earth's surface, including the leaf, its solar energy gets absorbed or transmitted or reflected back to the sensor. The images of reflected solar energy are called *spectral signatures*. The property that is used to quantify these spectral signatures is known as *spectral reflectance* (Lillisand and Kiefer, 2000; Govender *et al.,* 2007). The leaf spectral reflectance, which is the radiance reflected from the leaf expressed as a percentage of incident radiance through a range of spectrum wavelengths, highlights the change in spectral energy distribution of the reflected in relation to incident radiation (Carter, 1991). The results are given in a form of a graph of spectral reflectance of an object as a function of wavelength named *spectral reflectance or response curve* (Lillisand and Kiefer, 2000).

The spectral reflectance of a plant is governed by leaf structures, both external and internal structures, and its biochemical concentrations as well as its biological and biochemical reactions such as photosynthesis and transpiration taking place in the leaf (Xu *et al.,* 2007). However, plant anatomy also influences leaf optical properties as leaves from xeric environments can have higher reflectance in the shorter wavelengths due to higher contents of silicates in their leaves (Alvarez-Añorve *et al.,* 2008).

Although leaf structure varies from plant to plant, the following description provides a general outline of the main elements common to most plants with regard to remote sensing in vegetation or agricultural studies. With regard to external structure of the leaf, the cuticle and the upper epidermis are both transparent to the energy radiation hence very little radiation is reflected from the outer portion of the leaf (Campbell, 2002). This is illustrated in Figure 2.1.

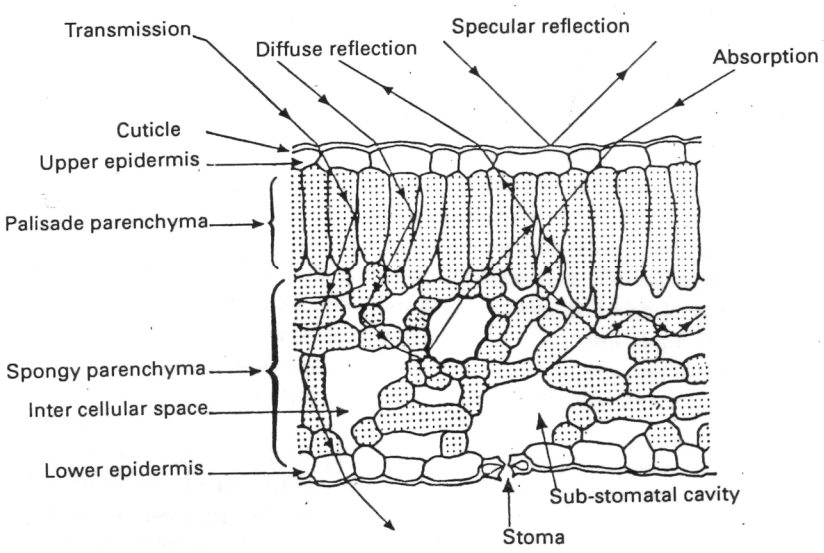


Fig. 2.1 Schematic representation of the interaction of irradiance (incoming radiation) with leaf tissues (Guyot, 1990, pp 21).

Below the upper epidermis, the leaf consists of palisade parenchyma or tissue characterized by vertically elongated cells arranged in parallel manner. These cells contain the largest number of chloroplasts, which are specialized lens-shaped structures containing chlorophyll. Chlorophyll, a green pigment fundamental to the light reaction to photosynthesis, appears in many forms in the leaf but most common in almost all plants are chlorophyll *a* and *b,* but all in all about ten forms were identified each with its unique absorption spectrum (Campbell, 2002; Kumar *et al*., 2003).

On the lower side of the leaf is lower epidermis which contains stomata, the main characteristic that makes it different from the upper epidermis. The stomata have guard cells which can open and close to allow or prevent movement of air, that is, carbon dioxide (CO_2) for photosynthesis, respiration and maintaining thermal balance, into the leaf. When the stomata are opened, moisture loss is minimized and maximum light transmission through the upper epidermis is experienced (Campbell, 2002). The whole electromagnetic spectrum range in relation to reflectance of healthy green leaf is illustrated in Figure 2.2.

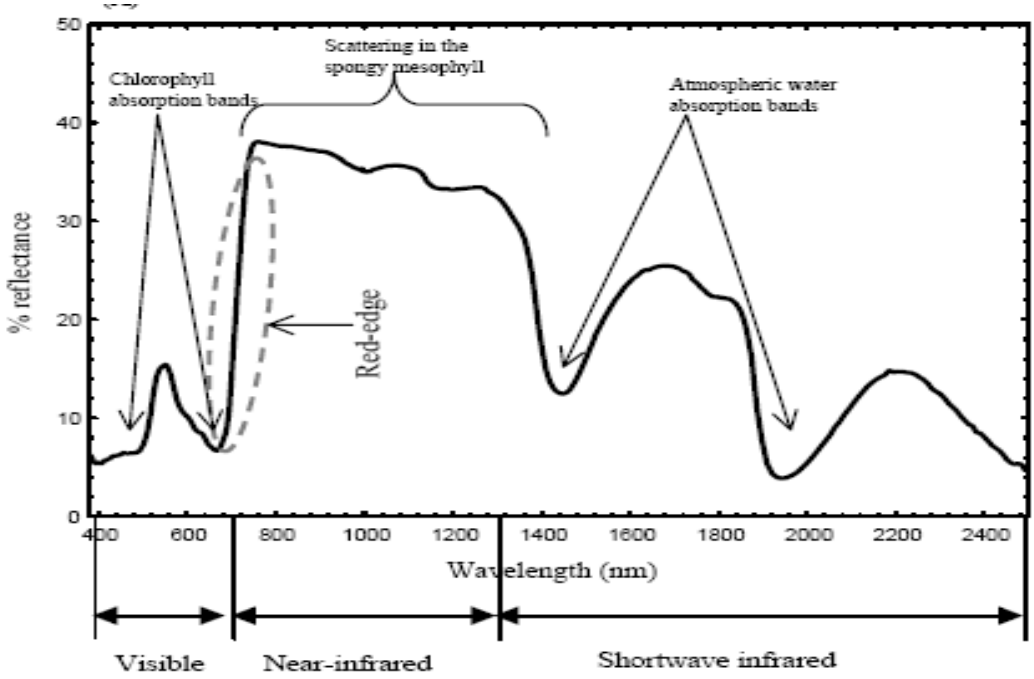


Fig. 2.2 Reflectance spectrum for healthy green leaf using handheld GER 3700 spectrometer (Cho, 2007, pp 4).

The visible portion (400 – 700 nm) of the electromagnetic spectrum is characterized by low leaf reflectance, (less than 15 percent of the energy incident) as well as low transmittance due to strong absorption by photosynthetic and accessory plant pigments such as chlorophyll, xanthophylls, carotenoids and anthocyanins (Figure 2.2) (Guyot, 1990; Kumar *et al*., 2003; Pinter *et al.,* 2003; Xu, 2007; Asner, 2008; Lucas *et al.,* 2008). Among these pigments, chlorophyll is the most crucial pigment. Chlorophylls (both chlorophyll *a* and *b*) absorb more energy radiation in the wavebands centered at about 450 nm (in the blue) and 670 nm (in the red) for photosynthesis, hence these wavebands are known as *chlorophyll absorption bands* (Guyot, 1990; Lillesand and Kiefer, 2000; Campbell, 2002; Cho, 2007; Tilling *et al.,* 2007; Asner, 2008; Lucas *et al.,* 2008).

From visible to Near Infrared (NIR) region of the reflectance spectrum, the spectral reflectance signature for healthy vegetation increases dramatically at around 700 nm, this is highlighted by dotted lines forming a circle around the spectral curve in Figure 2.2. This slope is known as the red-edge (Lillesand and Kiefer, 2000; Pinter *et al.,* 2003; Govender *et al.,* 2007). The red-edge is known by the low red chlorophyll reflectance to the high reflectance around 800 nm (known as red-edge shoulder) associated with leaf internal structure and water content (Dawson and Curran, 1998; Kumar *et al*., 2003). The spectral shift of the red-edge (670 - 780 nm) slope, which is the most studied portion of the spectral reflectance curve, is associated with leaf chlorophyll content, phenological state as well as plant stress (Kumar *et al*., 2003; Cho, 2007). As this slope is a fairly wide feature, the concentration is on the wavelength of maximum slope of the red-edge termed red-edge inflection point or red-edge position (REP) (Clevers and Jongschaap, 2001; Kumar *et al*., 2003; Imanishi *et al.,* 2004; Cho, 2007). If there are low chlorophyll concentrations in the leaf, these will cause the red-edge slope and REP towards the shorter wavelengths, hence the "blue shift", while high chlorophyll concentrations will cause shifts of the red-edge slope and REP towards longer wavelengths, resulting in the "red shift" (Clevers and Jongschaap, 2001; Imanishi *et al.,* 2004; Cho, 2007; Ismail *et al.,* 2008; Lucas *et al.,* 2008).

The NIR portion (700 – 1300 nm) of the spectrum is characterized by highest reflectance, ranging from 40 to 50 percent of the energy incident upon the target, while about 5 percent is absorbed and the rest is transmitted (Leblon, undated; Lillesand and Kiefer, 2000; Govender *et al.,* 2007). The leaf does not contain substances that absorb incoming radiation strongly in this portion of the electromagnetic spectrum (Carter, 1991; Xu *et al.,* 2007). The leaf optical properties are governed by leaf internal structures, thus scattering in the spongy mesophyll cells (Figure 2.2) (Lillesand and Kiefer, 2000; Campbell, 2002; Pinter *et al.,* 2003; Asner, 2008). Beyond the NIR, that is in the SWIR (from 1300 – 2500 nm), reflectance curve starts to go down with some absorption peaks caused by water absorptions at wavebands centered around 1400, 1900 and 2500 nm. These wavebands are often called *atmospheric water absorption bands* (Guyot, 1990; Lillesand and Kiefer, 2000; Kumar *et al*., 2003; ASD; 2006; Cho, 2007).

2.4.3 Remote Sensing of Sugarcane Crop

Even though some work have been undertaken on remote sensing of sugarcane worldwide (Hadsarang and Sukmuang, 2000; Schmidt *et al*., 2000; 2001; Gers and Schmidt, 2001; Bapel *et al*., 2003; Gers, 2003a; b; 2004; 2005; Apan *et al.,* 2004a; b; Galvão *et al.,* 2005; Fortes and Dematte, 2006; Xavier *et al.,* 2006; Lebourgeois *et al.,* 2007; Abdel-Rahman *et al.,* 2008a; b), none of this has strictly focused on the sugarcane optical properties.

Sugarcane (*Saccharum spp*. Hybrids) is a tall-growing perennial crop grown in tropical and subtropical regions (Muchovej *et al.*, 2005; Abdel-Rahman and Ahmed, 2008). Sugarcane crop is an extremely water intensive and is characterized by leaves, major contributors of light reflection from the crop, which consist of lamina and sheath. The lamina is an expanded part of a leaf (blade) and it hangs free from the main stalk of the crop to absorb sunlight, transpire water, and exchange Oxygen (O_2) and CO_2. The lamina is characterized by the lager vein in the center, known as midrib, and parallel veins interconnected by small lateral veins. The midrib contains larger conducting tissue hence it supports the lamina in the space around the cane plant. On the other hand the sheath wraps tightly around the main stalk of the crop and supports the lamina by a flexible collar known as dewlap (Muchovej *et al.*, 2005).

There is a general outline of the main elements common to most plants with regard to remote sensing in vegetation or agricultural studies. That is, the review of the whole electromagnetic spectrum range (350 – 2500 nm) in relation to reflectance of healthy green leaf. Sugarcane is also under that umbrella. Generally, the spectral reflectance of sugarcane plants is based on four factors, namely canopy architecture, foliar chemistry, agronomic parameters such as LAI, geometry of data acquisition and atmospheric conditions (Fortes and Dematte, 2006; Abdel-Rahman and Ahmed, 2008). Abdel-Rahman and Ahmed (2008) further stated that among these factors, canopy geometry seems to be the most important factor affecting spectral reflectance properties of sugarcane.

Spectral reflectance properties of sugarcane also depend on sugarcane phenological stages such as pre-emergence, emergence, tiller emergence and flowering. Among these stages, tiller emergence is of prime concern in remote sensing applications that rely on measurements of light energy reflected from sugarcane canopy. Flowering in sugarcane depends on temperature, sunlight and day length conditions and it does not occur regularly (Gers, 2003b).

However, there is a rising need for detailed studies of spectral optical properties of sugarcane leaves as this will make the applications of remote sensing in sugarcane agriculture quite understandable.

2.4.4 Spectral Vegetation Indices

Spectral vegetation indices (VIs) have been developed to reduce huge data to a single number to assess certain characteristics (Nilsson, 1995). These can be calculated from multispectral and hyperspectral data. In most studies in application of remote sensing in agriculture, VIs have served as the basis because they are well correlated with vegetation parameters such as green biomass, LAI, leaf gap fraction, N, and chlorophyll as well as plant stress (Nilsson, 1995; Casanova *et al.,* 1998; Hansen and Schjoerring, 2003; Pinter *et al.,* 2003; Cho, 2007). Spectral VIs are defined as mathematical transformations of vegetation reflectance into dimensionless measures which function as predictors of vegetation parameters (Elvidge and Chen, 1995; Cho, 2007).

Spectral VIs are formed from combinations of several values that are added or divided or subtracted, or multiplied in a way that they yield single values that indicate the vigor of vegetation within a pixel (Campbell, 2002). Researchers tend to use different spectral band combinations and names to distinguish between quantities or conditions. Spectral VIs include Ratio Vegetation Index (RVI), Normalized Difference Vegetation Index (NDVI), Photochemical Reflectance Index (PRI), Nitrogen Reflectance Index (NRI), Normalized Pigment Chlorophyll Index (NPCI), Red Edge Index (REI), Normalized Difference Red Edge Index (NDRE), Water Band Index (WBI), Water Band Ratio (WBR), Crop Water Stress Index (CWSI), Normalized Difference Index (NDI) and Carter Index (CI) (Leblon, undated; Jordan, 1969; Huete, 1988; Cater, 1994; Elvidge and Chen, 1995; Nilsson, 1995; Barnes *et al.,* 2000; Clevers and Jongschaap, 2001; van der Meer *et al.,* 2001; Campbell, 2002; Hansen and Schjoerring, 2003; Pinter *et al.,* 2003; Ferwerda *et al.,* 2005; Cho and Skidmore, 2006; Ray *et al.,* 2006; Tilling *et al.,* 2007; Cho, 2007; Meyer and Neto, 2008). Some of these VIs and their applications in agriculture are described in the next section.

2.5 Hyperspectral Remote Sensing in Agriculture

2.5.1 Imaging Spectroradiometry and Non-imaging Spectroradiometry

Interest in the application of hyperspectral data in agriculture is growing rapidly even though crop growth is dynamic and its monitoring is challenging (Bappel *et al.,* 2003). Hyperspectral remote sensing has been used interchangeably with spectroradiometry. The spectroradiometry is categorized into two types, namely; imaging and non-imaging spectroradiometry. Imaging spectroradiometetry involves use of spectroradiometers attached to remote sensing platforms such as airborne and spaceborne platforms. These provide images like multispectral sensors but with much higher spectral resolution. This high resolution feature makes it realistic for these systems to identify species and detect minor leaf biological and physiological changes that cannot be detected by multispectral scanners from air or space (Kumar *et al*., 2003). These include Moderate Resolution Imaging Spectroradiometer (MODIS), Airborne Visible/Infrared Imaging Spectrometer (AVIRIS), Hyperspectral Mapper (HYMAP), High Resolution Imaging Spectrometer (HIRIS), Multiangle Imaging Spectroradiometer (MISR), Medium Resolution Imaging Spectrometer (MERIS), and Hyperion (Johnson *et al.,* 1994; LaCapra *et al.,* 1996; Green *et al.,* 1998; Clevers, 1999; van der Meer *et al*., 2001; 2003; Kumar *et al*., 2003; Huang, *et al.,* 2004; Galvão *et al.,* 2005; Xavier *et al.,* 2006).

The forthcoming development of operational spaceborne imaging spectrometer missions, such as Hyperspectral Environment and Resource Observer (HERO) and Environmental Mapping and Analysis Program (EnMAP), as well as Sumbandila (ZASat-002, South African first satellite), will facilitate the development of a greater opportunity of practical applications of remote sensing. Simultaneously, there is a growing interest on the development in field-based sensors for application in precision agriculture (Scholes and Annamalai, 2006; Blackburn, 2007).

Non-imaging spectroradiometry on the other hand uses similar spectroradiometers with high spectral resolution except that they do not provide images and they are used on ground-based platforms. Non-imaging spectroradiometers provide most accurate data as they collect detailed spectral measurements from known features, and hence they are mainly used as reference or ground truth data for both airborne and spaceborne sensors (Aronoff, 2005). For this reason, a large number of ground-based studies have been undertaken for investigation of

the feasibility of utilizing hyperspectral data for vegetation studies. These include spectrometers and analytical spectral devices (ASD) spectroradiometers (Kumar *et al*., 2003; ASD, 2006).

This study focuses on the application of non-imaging spectroradiometer, a hand-held ASD spectroradiometer at leaf-level hence a thorough description of ASD spectroradiometer is of great importance. The hand-held *ASD Field Spec® 3* spectroradiometer is a specialized kind of spectrometer that measures spectral reflectance, spectral transmittance, spectral radiance, spectral irradiance and spectral absorbance using visible near-infrared (VNIR) and short-wave infrared (SWIR) spectra. However, all the above mentioned measurements can be done with the help of various set-ups and built-in processing of the radiance signal. The spectrum wavelength ranges from 350 – 2500 nm with spectral sampling interval of 1.4 nm for the region 350 – 1000 nm and spectral sampling interval of 2 nm for the region 1000 – 2500 nm (ASD, 2006). Its main features and advantages over other spectrometers are that accuracy and precision due to its high signal-to-noise ratio, transportability due to resistant to changes in temperatures as well as speed as 10 spectra per second can be measured for the entire spectrum range.

There is a fragile fibre optic cable bundle which brings light from the target object into the instrument and then the instrument will pass the information to the computer notebook where the spectral curves will be captured and saved for interpretation and further analysis. There is also a spectralon panel which is used for measuring a "white reference" reading which must reflect nearly 100 % of the light before any spectral measurements can be taken. This calibration or optimization process is done regularly, after every 10 – 15 minutes. The next sections will present a brief overview of applications of both imaging and non-imaging spectroradiometers on agriculture.

2.5.1.1 Air and Space-borne Level Application

Hyperspectral imaging or imaging spectroradiometry is a powerful as well as versatile tool for continuous sampling and selecting narrow wavebands that are sensitive to specific crop variables, such as plant diseases, pests, nutrients and environmental stresses (Nilsson, 1995; Hansen and Schjoerring, 2003). However, sometimes satellites pass over a target region too early in the morning for capturing an image hence measurements are made over canopy that

still has dew on its leaves and other times after the dewfall has dried, this makes it difficult to compare data accurately as dew has a profound influence on spectral reflectance. Airborne spectroradiometers have an advantage over these satellites as suitable time for measurements, height for measurements, calibrations, spectral/spatial resolutions and acceptable weather conditions can be selected (Cetin, undated; Nilsson, 1995).

The Airborne Visible/Infrared Imaging Spectrometer has been used significantly in agricultural crops in recent years and it was the first hyperspectral sensor to measure the solar energy reflected spectrum from 400 nm to 2500 nm at 10 nm intervals (Green *et al.,* 1998). Most applications of AVIRIS in agricultural crops have been focused on foliar chemistry (Johnson *et al.,* 1994; LaCapra *et al.,* 1996; Clevers, 1999). For instance, LaCapra *et al.* (1996) used multiple linear regression (MLR) to develop calibration equations for N and lignin concentrations based on AVIRIS reflectance of rice from five fields in California. LaCapra *et al.* (1996) found that calibration equations from MLR of AVIRIS can be used to predict N concentration in rice even though it was not easy to develop general equation as the MLR calibration equations were based on a different set of wavelengths for each subset of data.

Jago *et al.* (1999), Bappel *et al.* (2003) and Zarco-Tejada *et al.* (2004) tested the utility of Compact Airborne Spectrographic Imager (CASI) in monitoring agricultural crops condition, winter wheat and rice. Jago *et al.* (1999) used both field based (Geophysical Environmental Research (GER) IRIS Mark IV, a dual field-of-view spectroradiometer) and airborne based (CASI) data from a winter wheat field site under different levels of N fertilization in the United Kingdom (UK) to derive a relationship between REP and canopy chlorophyll concentration. The results showed strong correlation between REP and chlorophyll concentrations in both field and airborne data, hence it was concluded that REP can be used to estimate chlorophyll concentration which indicates that remote sensing techniques can be used for inferring grain yield. Bappel *et al.* (2003) studied spectral indices, Photochemical Reflectance Index (PRI) from CASI data over sugarcane sites of Reunion Island as bio-indicators of crop condition. Bappel *et al.* (2003) found out that hyperspectral data can estimate bio-indicators of sugarcane crop conditions as biomass and N correlated highly with CASI reflectance (R^2 = 0.78 and 0.65, respectively). Bappel *et al.* (2003) indicated that the establishment of N – sugar concentration – leaf water content for the same data was on the way.

van der Meer *et al.* (2001) studied the performance of MERIS relative to the scale of observation using simulated datasets on different forests and bare agricultural field in France. Even though they used many VIs such as NDVI, Perpendicular Vegetation Index (PVI) and Soil-adjusted Vegetation Index (SAVI), the results demonstrated that correlation between biomass and NDVI for MERIS simulated datasets was better, however it was modest.

Apan *et al.* (2004a) and Galvão *et al.* (2005) discriminated sugarcane varieties using discriminant analyses and spectral indices from EO-1 Hyperion hyperspectral data, the first orbital spaceborne hyperspectral sensor, in Australia and Brazil, respectively. From Apan *et al.* (2004a), the results indicated high discrimination between sugarcane varieties, with accuracy of above 74% from discriminant analysis, and for the spectral indices, best results were from those indices related to leaf pigments and the leaf internal structure. However, for the classification of the entire Hyperion image, the accuracy was low which could be due to non-image information such as crop calendar, soil background and leaf geometry. Therefore Apan *et al.* (2004a) suggested that this non-image information should be considered for improved classification accuracy. Galvão *et al.* (2005) also found that best spectral indices for discrimination between varieties were those indices related to leaf pigments, such as chlorophyll content and the leaf internal structure as well as water content. In addition, the comparison of ground truth reference data with classified image derived from discriminant analysis confirmed best performance of the discriminatory model. However, it was suggested that further research on other areas should be carried out to validate the results.

Xavier *et al.* (2006) performed sugarcane crop classification with MODIS using multi-temporal Enhanced Vegetation Index (EVI) in São Paulo State, Brazil. Xavier *et al.* (2006) discovered that the use of cluster analysis in an unsupervised classification can be used to distinguish sugarcane from natural vegetation, urban areas, annual crops, water bodies as well as some patterns. On the other hand, pasture which seemed to have similar temporal EVI with sugarcane became problematic. However, the confusion from pasture could be solved using images from higher spatial resolution sensors accompanied by sugarcane classification procedure. Supervised classification was difficult due to both large planting and large harvesting periods hence it should be pursued for the whole State.

2.5.1.2 Canopy Level Application

Vegetation canopies are composed of many leaves which may differ in terms of size, orientation, shape, structure as well as coverage of the ground surface (Campbell, 2002). Canopy reflectance spectra are affected by many factors in addition to the ones mentioned above, these include internal factors such as soil color, canopy geometry, row orientation, and optical properties of other plant parts such as flowers and fruits, and external factors such as solar elevation, orientation and inclination of the view axis, and atmospheric conditions such as wind speed (Guyot, 1990; Nilsson, 1995; Abdel-Rahman and Ahmed, 2008; Asner, 2008).

The upper leaves shadow the lower leaves hence reflectance from the lower leaves is affected by that shadow (Campbell, 2002). As canopy reflectance is a combination of reflectance spectra of the plants and underlying soil, it is governed by some vegetation parameters such as LAI. Thus, as LAI increases, the contribution of the soil or background to the resulting reflectance decreases and the multiple scattering of light caused by plant cells increases (Guyot, 1990; Yoder and Pettigre-Crosby, 1995; Asner, 1998; Ray *et al.,* 2007). In addition, the impact of these effects varies with wavelengths (Yoder and Pettigre-Crosby, 1995). The NIR reflectance increases proportionally to the number of layers of leaves in a canopy reaching maximum reflection at about eight layers of leaves as a result of reflectance increase from the spongy mesophyll (Lillesand and Kiefer, 2000; Alvarez-Añorve *et al.,* 2008). Then afterwards, subsequent addition of canopy leaves reduces NIR reflectance, given that shadowing traps incoming light energy (Alvarez-Añorve *et al.,* 2008). This further reduces reflectance in the SWIR due to increase in canopy moisture content caused by shadowing (Alvarez-Añorve *et al.,* 2008). For applications of spectroradiometers at canopy level, fore optic pistol grips are normally mounted on either ground-based platforms such as tripods, ladders and trucks, thus over 1 m above crop canopy (Nilsson, 1995; ASD, 1999; Tilling *et al.,* 2007). However, airborne and spaceborne levels are also regarded as canopy level by most scientists as their reflectance spectra are affected by similar factors as canopy reflectance spectra.

Mutanga *et al.* (2003) investigated the potential of high-resolution reflectance using GER 3700 spectroradiometer to discriminate differences in N concentration of *Cenchrus cliaris* grass in the greenhouse under different fertilization treatment at canopy level in the Netherlands. The findings showed that there were statistically significant differences in

canopy spectral reflectance between treatments within certain wavebands. Continuum-removal in the visible region between 550 and 750 nm was further used to detect the effect of varying N supply. Results illustrated that high N treatment had deeper and wider absorption pits than both low N treatment and the control (no N). Overall, the results indicated that hyperspectral remote sensing can be used for classification and mapping of pasture quality, hence grasslands with different levels of nutrients.

Xue *et al.* (2004) assessed the potential of canopy level reflectance to determining N status in rice (*Oryza sativa L.)* in USA. A portable ground MSR16 radiometer (CROPSCAN, Rochester, MN) was used for acquiring canopy spectral reflectance over the wavelength range of 447 to 1752 nm. The results indicated that the ratio index of NIR to green (R_{810}/R_{560}) was linearly correlated with total leaf N accumulation, independent of N level and growth stage. Therefore, the conclusion was that this ratio index should be used for nondestructive monitoring of N status in rice.

2.5.1.3 Leaf Level Application

Unlike canopy level, for leaf level, the effect of other factors, such as other plant parts, is reduced or controlled as the fibre optic probe is pointed exactly on the target leaf. Hence the percentage reflectance of a single leaf is higher than that of canopy. Leaf level applications provide most accurate data as they collect detailed spectral measurements from known features, and hence they are mainly used as reference or ground truth data for both airborne and spaceborne level applications (Aronoff, 2005). Applications of hyperspectral remote sensing at leaf level or scale are discussed in the next sections. The focus will be mainly on nutrient detection and pest/disease identification.

2.5.2 Hyperspectral Remote Sensing of Foliar Chemistry

The dynamics of plant pigments have strong relation with the physiological status of plants, hence information concerning temporal and spatial variations of pigments can be a valuable indicator of a range of key properties and processes in agricultural crops (Blackburn, 2007). Foliar chemical composition is very important as it can provide information about nutrient cycling and plant stress and can also provide information about the ecosystem's processes, as

well as input to ecosystem simulation models (Mthembu, 2006). Therefore, remote sensing of foliar chemistry can help with providing such information at different scales.

2.5.2.1 Estimation of N, Si and Other Nutrients

The potential to estimate the nutrient status in important agricultural crops such as maize and sugarcane is of significant interest (Ferwerda and Skidmore, 2007). Estimates of the chemical concentrations, such as chlorophyll, lignin, N, water content, of agricultural and forestry canopies can be made using hyperspectral remote sensing (Koklay and Clark, 1999; Curran, 2000; Ahmed, 2006). In general, the measurement of plant biochemical concentrations by remote sensing is a complex problem (Koklay and Clark, 1999). The use of high spectral resolution data creates the chance to select the optimal wavebands for prediction of plant chemical properties such as chlorophyll and N (Ferwerda *et al.,* 2005). Nitrogen is one of the most important and or crucial elements which determine quality and health in plants (Johnson, 2001; Mutanga *et al.,* 2003). However, chlorophylls contain large amounts of total leaf N hence chlorophyll concentration can provide an accurate indirect assessment of plant N status, and chlorophyll is highly correlated to leaf N (Yoder and Pettigrew-Crosby, 1995; Mutanga *et al.,* 2003; Blackburn and Ferwerda, 2008; Asner, 2008; Lucas *et al.,* 2008).

Hyperspectral remote sensing with its narrow and sensitive wavebands has the potential to estimate this ratio, thus leaf N and Si concentrations in sugarcane. However, none of studies have examined the opportunities for quantifying leaf Si concentration from reflectance spectra. Some of studies examining the opportunities for quantifying leaf N concentrations using hyperspectral remote sensing are presented below.

Ayala-Silva and Beyl (2005) investigated the effects of nutrient deficiencies, N, Phosphorus (P), K, Calcium (Ca) and Magnesium (Mg) on spectral reflectance properties of wheat leaves under growth chamber and greenhouse in USA. The spectral measurements were collected using a Spectronic 601 spectrophotometer. Results showed that all macronutrient deficiencies tested affected chlorophyll content by reducing it and increased reflectance in the visible and IR ranges and also caused a shift in the position of the red-edge towards shorter or longer wavelengths depending on the element. Results illustrated that N and Mg deficiencies had the most pronounced effect on chlorophyll height and leaf reflectance. A conclusion was that spectral measurements are useful for detecting early nutrient deficiencies in wheat if the

specific element deficiency is known. However, distinguishing among individual nutrients could be problematic.

Zhao *et al.* (2005) studied the effects of N deficiency on sorghum growth, physiology and its leaf reflectance properties in Mississippi, USA using a hand-held ASD *Field Spec Pro FR* Spectroradiometer. The results indicated that N deficiency significantly reduced leaf chlorophyll content and photosynthetic rate (P_n), resulting in lower biomass production. Its effect on reflectance was that it increased leaf reflectance at 555 and 715 nm and caused a red-edge shift to the shorter wavelength. Leaf N and chlorophyll were also linearly related to ratios of R_{405}/R_{715} ($R^2 = 0.68$) and R_{1075}/R_{735} ($R^2 = 0.64$), respectively, as well as the first derivative of the reflectance ($dR/d\lambda$) in the red-edge around 730 or 740 nm ($R^2 = 0.73 – 0.82$). It was therefore concluded that the specific reflectance ratios or the first derivative offer opportunity of hyperspectral remote sensing to estimate leaf chlorophyll and N status in sorghum rapidly and non-destructively.

Abdel-Rahman *et al*. (2008a) investigated the potential of hyperspectral remote sensing using a hand-held ASD *Field Spec® 3* spectroradiometer with the spectrum range of 350 – 2500 nm in estimation of sugarcane leaf N concentration in South Africa. The spectral measurements were taken on leaf samples from variety N19 of two age groups (4 – 5 and 6 – 7 months) under controlled conditions. Abdel-Rahman *et al*. (2008a) used correlation and regression to determine the relationship between N concentration and first-order reflectance throughout the spectrum range and wavebands which showed strongest relationship were used to develop spectral indices. Results highlight that for the 4 – 5 months cane, the R_{744}/R_{2142} index was used to estimate N concentration ($R^2 = 0.74$) while the modified NDVI (($R_{2200} - R_{2025}$)/ ($R_{2200} + R_{2025}$)) was linearly related to N concentration ($R^2 = 0.87$) on 6 – 7 months cane.

2.5.2.2 Water Stress and Status

In agricultural crops it is important to be in the position to detect the onset of water stress as early as possible so that some preventive measures like irrigation can be considered (Kumar *et al*., 2003). Water availability is a critical factor in plant survival and development and water stress is one of the most common limitations of primary productivity (Kumar *et al*., 2003). In some crops, drought conditions can lead to increase of some pest and disease infestations. For instance, in sugarcane it is well known that water stressed plants are very susceptible to attack

and population build up of *E. saccharina* as dry or dead leaves are habitat for laid *E. saccharina* eggs (Atkinson and Nuss, 1989; Atkinson *et al.,,* 1989; Gers, 2004). This can be regarded as one of the secondary effects of water stress. The spectral quality of light energy reflected from plant leaves has long been depended upon as an indicator of plant stress (Carter and Knapp, 2001). Hence remote sensing is the best technology for monitoring of crop growth at a large scale. Generally, early detection of plant stress by remote sensing depends largely on identifying the spectral portions in which plant reflectance is most responsive to unfavorable growth conditions (Carter and Miller, 1994).

Carter (1991) investigated primary (from radiative properties of water) and secondary (could not be explained by radiative properties of water, such as leaf pigments) effects of water content on leaf spectral reflectance in Mississippi, USA, across six plant species, including cane-grass. The study used a scanning radiometer (IRIS, GER, Milbrook, NY) for spectral measurements. Results illustrated that decreased water content of the leaf generally increase reflectance throughout the whole spectrum range (400 – 2500 nm). However, the sensitivity of reflectance to water content was greatest in the water absorption bands around 1450, 1940, and 2500 nm. Secondary effects resulted from pigments as the sensitivity maxima also occurred between 400 and 720 nm.

Ray *et al.* (2006) evaluated various hyperspectral indices from ASD *Field Spec Pro 2000* hand-held spectroradiometer data for estimation of LAI and potato crop discrimination under various water irrigation treatments in India. Results indicated that hyperspectral indices were better than LAI in the detection of the differences among crops under various water irrigation treatments. A set of five most optimum bands for discrimination of the potato crops under three irrigation treatments was produced from the discriminant analysis.

2.5.3 Hyperspectral Remote Sensing of Crop Pests and Diseases

Plant pests and diseases cause serious economic losses in yield and quality of commercial crops, hence the detection, monitoring and assessment of their symptoms is essential (Apan *et al.,* 2005; Datt *et al.,* 2006). Pathogens and pests can induce physiological stresses and physical changes in plants which can directly or indirectly affect reflectance properties of plants. Therefore, this makes it realistic to use remote sensing techniques to assess pest and disease stresses as they are not biased and time consuming like traditional or visual

assessment and they can be used repeatedly as they collect measurements non-destructively (Nilsson, 1995; Apan *et al.,* 2005; Datt *et al.,* 2006; Mirik *et al.,* 2006a). Several studies have been undertaken on the use of hyperspectral remote sensing in the detection and monitoring of pests and diseases in agricultural crops, some of these studies are described below.

Apan *et al.* (2004b) used discriminant analyses and spectral indices from EO-1 Hyperion hyperspectral data to discriminate sugarcane areas affected by 'orange rust' (*Puccinia kuehnii*) disease in Australia. Forty spectral indices related to leaf pigments, the leaf internal structure as well as water content were generated. The results indicated that the discriminant function allowed ranking of each spectral index based on their ability to distinguish rust-affected from non rust-affected sugarcane. The results indicated that the combination of VNIR and moisture sensitive band (1660 nm) yielded maximum discrimination of rust affected sugarcane areas. Results further indicated the key role played by SWIR wavebands (1660 – 2200 nm) in discrimination of healthy and orange rust diseased cane crops. Therefore it was recommended that a follow-on study on detection of rust disease at various levels of severity using Hyperion would yield more information on the application of hyperspectral remote sensing in crop protection.

Mirik *et al.* (2006a) investigated the ability of digital image (camera) and reflectance (Ocean Optics S2000 hyperspectral hand-held spectrometer) data in quantification of greenbugs on winter wheat in USA. Mirik *et al.* (2006b) further used the same hyperspectral hand-held spectrometer and a Cropscan multispectral field radiometer to quantify aphid density (greenbug and bird cherry-oat aphid) in winter wheat. Results from both studies demonstrated that remotely sensed data recorded by hyperspectral spectrometer appeared functional in monitoring aphid population in winter wheat production under field conditions.

Apan *et al.* (2005) tested the potential of using hyperspectral remote sensing in the detection of the incidence of pests and diseases in vegetable crops, tomato and eggplants using a hand-held ASD *Field Spec Pro FR* Spectroradiometer in Australia. Spectral measurements of diseased/infested and healthy leaves were collected separately from both crops, tomato affected by fungal early blight disease (*Alternaria solani*) while eggplants had leaf holes caused by the 28-spotted ladybird (*Epilachna vigintioctopunctata*). Results demonstrated that hyperspectral measurements can be used to detect effects of pests and diseases in vegetable crops. The significant spectral bands for tomato disease estimation showed good relation with

the red-edge and visible as well as small portion of NIR wavelengths. The NIR region was found to be as equally significant as red-edge in prediction of eggplant's insect infestation using the regression model.

Datt *et al.* (2005) used the same hand-held spectrometer as above, to investigate the feasibility of imaging spectroscopy for early detection of pests and diseases in selected vegetable crops. Leaf and canopy spectral measurements were performed for both healthy and diseased/infested selected crops. The results showed clear separation between healthy and diseased crops but they went further to use first derivative reflectance to improve their results. The derived indices from these derivatives based on bands sensitive to pest/disease infestation and these indices provide a simple method for quantification of the level of disease activity within the range from healthy to severely infested crops. Both studies found that the use of hand-held field spectrometers provide a means of rapid observation and digital recording of many plant samples within a short time scouting through the fields, and hence this, in conjunction with Global Positioning Systems (GPS), can be used for field map creations by spatial interpolation among the sampling points.

Abdel-Rahman *et al*. (2008b) tested the ability of hyperspectral data in identifying and monitoring of damage caused by sugarcane thrips *Fulmekiola serrata* (Kobus) (Thysanoptera: Thripidae) in South Africa. A hand-held ASD *Field Spec® 3* spectroradiometer with the spectrum range of 350 – 2500 nm was used to capture spectral measurements on healthy and thrips-damaged cane from two varieties (N19 and N12) at leaf-level. Abdel-Rahman *et al*. (2008b) found out that there were significant differences in leaf reflectance with increasing thrips damage levels, with the red-edge region giving the highest statistically significant differences. It was then assumed that thrips induced chlorophyll and N deficiencies hence the highest significant difference was in the red-edge region.

Although many studies have been conducted on remote sensing of crop protection, none have been focused on the detection of *E. Saccharina* pest. Pests and pathogens can induce differences in spectral responses of plant leaves as they change the physiological responses to nutrient and environmental stress, biochemistry, and biophysical properties of leaves (Apan *et al.*, 2005). Reflectance based remote sensing techniques for pest identification capitalizes on the fact that most pests affect the outwards appearance of a plant in a particular manner either within the visible or outside the visible spectrum (Abdullah and Umer, undated). *Eldana*

Saccharina pest is not an exception, though its symptoms or effects are known to occur on cane stalks not on leaves. Therefore this study focused on investigating the potential of hyperspectral remote sensing in detection of sugarcane prone to attack or already attacked by E *Saccharina.*

2.6 Summary

This chapter focused on the review of *E. saccharina* pest as well as its economic negative impact in South African sugarcane production. It was noticed that *E. saccharina* causes losses of about R250 million per annum, and measures used to reduce the incidence of this pest were also highlighted. However, it was stated that there is no single measure that can provide an answer to the *E. saccharina* problem, hence only IPM, which is a combination of selective control measures that can work at appropriate times in the cane crop or pest cycle.

It was revealed in this chapter that remote sensing technology can be used to provide both quantitative and timely information on agricultural crops during their growing season. Remote sensing techniques have been used as potentially important tools for the identification of nutrient content, chlorophyll content, detection of pests and diseases, water stress, mapping, precision farming, hail damage, crop inventory as well as yield estimation in agricultural crops over wide areas with the ability to evaluate information in an unbiased way.

There are many factors affecting leaf optical properties such foliar chemistry, water content, leaf structure, thus both external and internal structure. Although leaf structure varies from plant to plant, the general outline of the main elements common to most plants with regard to remote sensing in vegetation or agricultural studies was presented. For foliar chemistry, photosynthetic and accessory plant pigments such as chlorophyll, xanthophylls, carotenoids and anthocyanins were discussed. Among these pigments, chlorophyll is the most crucial pigment. Chlorophylls contain large amounts of total leaf N hence chlorophyll concentration can provide an accurate indirect assessment of plant N status. The spectral shift of the red-edge (670 - 780 nm) slope is the most studied portion of the spectral reflectance curve, it is associated with leaf chlorophyll content, phenological state as well as plant stress.

A brief description of sugarcane crop and its spectral properties were also highlighted. Sugarcane crop is an extremely water intensive and is characterized by leaves, major

contributors of light reflection from the crop, which consist of lamina and sheath. Generally, the spectral reflectance of sugarcane plants is based on four factors, namely canopy architecture, foliar chemistry, agronomic parameters such as LAI, geometry of data acquisition and atmospheric conditions. Spectral reflectance properties of sugarcane also depend on sugarcane phenological stages such as pre-emergence, emergence, tiller emergence and flowering.

Both imaging spectroradiometry and non-imaging spectroradiometry applications showed the potential of hyperspectral remote sensing in providing information rapidly and non-destructively for monitoring of agricultural crops which will enhance the overall productivity. However, these studies focused mostly on the quantification of chlorophyll and N concentration of plant leaves from reflectance data. The studies presented in this chapter highlighted that hyperspectral can be used to monitor and detect pests and diseases in agricultural crops. For instance, pests and diseases can induce differences in spectral responses of plant leaves as they change the physiological responses to nutrient and environmental stress, biochemistry, and biophysical properties of leaves.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

The main aim of this study was to determine the potential use of hyperspectral data (spectroradiometry) for identifying sugarcane that is infested by *E. saccharina.* This chapter describes how this main aim has been achieved through laboratory chemical analyses, *in situ* spectral measurements and statistical analyses.

3.2 Experiment, Events and Measurements

3.2.1 Design

An on-going N x Si x variety trial taking place under shade house at SASRI was designed to study the combined influence of N and Si nutrients on *E. saccharina* infestation in different varieties (designed by Nikki Sewpersad (SASRI-Biometrician)). Seedcane materials of five normally grown varieties in South African sugarcane region that are resistant (N17and N21), intermediately-susceptible (N25 and N37) and susceptible (N14) to *E. saccharina* were collected and prepared for pre-germination. However, this study only focused on intermediately-susceptible and susceptible varieties (N14, N25 and N37) to *E. saccharina* (see Appendix A).

The major reason for choosing specifically N and Si is by stating that N and Si play important roles in the resistance and susceptibility of a range of crops to stalk borer *(E. saccharina)* damage (Meyer and Keeping, 2005a; b). For instance, high Si contents interfere in the feeding of *E. saccharina* larvae by damaging their mandibles (Savant *et al.,* 1999; Kvedaras *et al.* 2007). High N levels on the other hand are linked with high *E. saccharina* infestations because of shortened development time of the insect (Atkinson and Nuss, 1989). On the other hand, stress causes a redistribution of N from the leaves and growing shoot to the stalk where *E. saccharina* larvae feed (Atkinson and Nuss, 1989), therefore there might be reduction in leaf N concentration. In addition, recent studies show that N/Si ratio is correlated with *E. saccharina* damage and hence sugarcane with N/Si ratio greater than 2 are associated with

increasing risk of *E. saccharina* borer damage (Meyer and Keeping, 2005a), but this can be utilized mostly for modeling of *E. saccharina* potential outbreaks.

Pots containing clean, sieved and thoroughly leached river sand allowing precise control of nutrient supply were established in an outdoor sugarcane trial. The pots were arranged in a randomized split plot design with N*Si treatment as a whole plot treatment and variety as a split plot treatment. There were two replications; these resulted in 54 pots for the whole trial. Three N treatment levels were applied as ammonium sulphate (N1 = 30 ppm, N2 = 60 ppm and N3 = 90 ppm) via nutriculture (hydroponic) solutions added to different pots, while three Si treatment levels were applied and incorporated thoroughly into the sand of each pot as calcium silicate (Calmasil) according to the treatment plan (Si0 = 0 ppm, Si1 = 100 ppm and Si2 = 200 ppm) (see Appendix A). Germinated seedcane materials were transplanted into the pots as per treatment plan (see Appendix A). Fresh irrigation water containing 2 litres of nutrient stock solution and ammonium sulphate were applied after every 7 days (every Friday) using Hygrotech Seedling Mix., except in rainy days.

3.2.2 Spectral Measurements

Spectral measurements were undertaken using hand-held *ASD Field Spec® 3* spectroradiometer (ASD, 2006).

3.2.2.1 Foliar Chemistry

At the age of 3 months, the first leaf spectral measurements were undertaken by pointing the ASD fibre optic with bare fibre (23°) field of view (FOV) at the distance of 10 cm to the 3rd leaf (as this leaf contains most of the plant nutrients, N and Si) of the main plant in each targeted pot throughout the whole experiment on sunny days. The purpose of these measurements was to determine leaf reflectance variations as influenced by variety, N and Si treatments. The dark current and white reference were taken every 10 minutes, as well as less than 10 minutes where necessary, to account for unstable atmospheric conditions and sun angle with time of day (Datt *et al.,* 2006) and also to account for a variation in spectral response of plants with time (Mutanga *et al*., 2003). Notes were made to record anything of concern such as weather conditions and mistakes on spectral measurements.

When cane was 7 months old, the second leaf spectral measurements were undertaken at the 3rd leaf of the main plant in each targeted pot with similar purpose of determining if leaf reflectance variations were influenced by variety, N and Si treatments. The scanned 3rd leaf and two more 3rd leaves from each pot were taken for chemical (N and Si) analyses. The midribs were removed from the leaves and the leaves were oven dried at 70 ^{o}C for 24 hours. Removal of the midrib from the leaf blade is a standard practice for sugarcane foliar analysis (Ezenzwa *et al.,* 2005). The oven dried leaves were ground and 0.5 g were weighed for Si determination while 0.25 g were kept for N determination per sample or pot. Actual Si concentrations were determined by the dry ashing method while actual N concentrations were determined using manual Kjeldahl method (Horneck and Miller, 1998).

3.2.2.2 Water Stress and Status

At the age of 9 months, the sugarcane trial (using same design) was transferred to a shade house with transparent polycarbonate roofing and walls of green 40% shade cloth in preparation for subjecting the plants to water stress and inoculation with *E. saccharina* eggs. Once the plants were in the shade house, the N supply was terminated, and excess stalks/tillers (<1 m) were removed, keeping maximum of 5 stalks per pot. The process of inducing water stress was also initiated. The irrigation schedule to induce water stress was as follows: 1st week = 1 litre (10 minutes) per pot per day; 2nd week = 0.7 litres (7minutes) per pot per day; 3rd week = 0.5 litres (5 minutes) per pot per day and; 4th week = 0.3 litres (3 minutes) per pot per day till harvest. The third spectral measurements were made three weeks after the plants have been subjected to water stress (when cane was 10 months old) and a few hours before *E. saccharina* inoculation. The two more 3rd leaves from each pot were taken for chemical (N and Si) analyses. The scanned 3rd leaves from each pot were cut and weighed immediately to obtain fresh leaf weight. Then they were taken to the laboratory where they were oven dried at 105 ^{o}C overnight. Oven dried leaves were weighed individually to obtain dry leaf weight. The scanned 3rd leaves were combined with the other two 3rd leaves taken from each pot for chemical (N and Si) analyses after removal of midribs. Then Absolute Water Content (AWC) for each leaf was calculated as follows:

$$\%AWC = [(\text{Fresh Leaf Weight} - \text{Dry Leaf Weight})/ \text{Fresh Leaf Weight}] * 100 \quad [\text{Eq } 3.1]$$

Then the plants were inoculated with *E. sachharina* eggs (placed on tissue paper in a batch of 100 eggs) on the lower base of one stalk. Since the number of stalks varied per pot, pots with 3 or less stalks were inoculated with 100 eggs (1 batch) while those with more than 3 stalks were inoculated with 200 eggs (2 batches) per pot. The infestation was allowed to progress for 2 months.

3.2.2.3 *E. saccharina* Detection

At harvest (12 months cane age), the last spectral measurements were taken. The same procedures as on the third spectral measurements, water content determination and chemical analyses were followed. In addition, stalks were collected from the targeted pots and split longitudinally for recording the number of stalks damaged by *E. saccharina* and for counting the number of *E. saccharina* larvae and *E. saccharina* pupae in each pot. Borer damage was measured as percentage of stalks bored or damaged by *E. saccharina* larvae (% Stalk Damage or % Damage) (see Equation 3.2) while borer performance was measured by taking the total number of larvae and pupae found in the stalks (Number of *E. saccharina*) (see Equation 3.3) (Mutambara-Mabveni, 2007; Way and Goebel, 2007).

$$\%\ \text{Stalk Damage or \% Damage} = [(\text{Total number of stalks bored by Eldana})/ (\text{Total number of stalks per pot})] * 100 \quad [\text{Eq 3.2}]$$

$$\text{Number of } E.\ saccharina = \text{Total number of larvae} + \text{Total number of pupae} \quad [\text{Eq 3.3}]$$

3.3 Spectral Data Pre-processing

ViewSpec software (ASD, 2006) was used for viewing graphic reflectance results as well as reflectance data pre-processing such as averaging spectra to reduce within leaf variability and to increase statistical power, performing first derivatives and exporting spectra into American Standard Code for Information Interchange (ASCII) text files which were easily imported into a statistical package. The first derivative spectra were performed mainly to reduce effects of multiple scattering of radiation due to sample geometry and surface roughness, and to locate the positions of absorption features and inflection points on the spectra (Datt *et al.,* 2006). Noisy wavebands due to water absorption features and sun-angle effects, e.g. around 1400

nm, 1900 nm and 2500 nm, were identified and thus excluded from the analysis except for estimation of water content or stress.

3.4 Statistical Data Analysis

3.4.1 Laboratory Samples

Factorial ANOVA was performed to determine if there were significant differences in dependent variables (N concentration, Si concentration, N/Si ratio, *E. saccharina* damage, and water content) caused by different factors (N treatment, Si treatment, and variety) as well as their interactions with regard to cane age. A correlation matrix was also performed to determine the relationship between all the above mentioned variables, with major concern on N/Si ratio, water content and *E. saccharina* damage as the first two (N/Si ratio and water stress) are the major factors affecting *E. saccharina* infestation or damage.

3.4.2 Leaf Reflectance

One-way ANOVA was performed to determine whether there were significant differences in the reflectance spectra caused by different factors (N treatment, Si treatment, and variety) at each wavelength at different cane ages.

The damage induced by *E. saccharina* on sugarcane stalks ranged from 0 – 100% stalk damage (see Equation 3.2). Therefore, in order to discriminate between healthy and *E. saccharina* damaged cane using leaf reflectance, 3 spectra of the third leaf of the main stalk from each pot were taken, using same distance and FOV indicated in section 3.2.2.1. The three spectra were then averaged to give a single representative spectrum per pot. This averaging was done to reduce within leaf variability (ASD, 2006). Then the damage range (0 – 100%) was categorized into 2 levels: healthy (0% stalk damaged), and damaged cane (1 – 100% stalk damaged). The numbers of pots in each damage level were as follows, 6 pots for healthy and 48 pots for damaged cane. Then ANOVA was conducted to determine if leaf reflectance can significantly discriminate healthy cane from *E. saccharina* damaged cane. Incidentally, this ANOVA is equivalent to T Test.

The damage range (0 – 100%) was further categorized into four levels: healthy (0% stalk damaged), low damage (1 – 39% stalk damaged), medium damage (40 – 69% stalk damaged) and severe damage (70 – 100% stalk damaged), to distinguish between various damage levels. The numbers of pots in each damage level were as follows, 6 for healthy, 11 for low damage, 22 for medium damage, and 15 for severe damage. For each pot, one leaf (from main stalk) was selected. Three spectral measurements were made on the leaf using same distance and FOV indicated in section 3.2.2.1 and were averaged to arrive at a single representative spectrum per pot. This averaging was done with purpose of reducing within leaf variability (ASD, 2006). Then an ANOVA was performed to detect if there were significant differences in the leaf reflectance spectra caused by different *E. saccharina* stalk damage levels. This was done for all varieties as well as for N37 on its own which was the most damaged variety.

To determine best hyperspectral wavebands, correlation matrices using Pearson's moment Product as well as linear regressions were performed to check the relationships between leaf reflectance as well as first derivative and all dependent variables (N, Si, N/Si ratio, water content, *E. saccharina* damage and number of *E. saccharina*) at different ages.

3.5 Spectral Data Analysis

3.5.1 Red-edge Region

The red-edge (670 – 780 nm) region of the electromagnetic spectrum was assessed to monitor changes in the red-edge slope and red-edge maximum inflection point or REP for variables (*E. saccharina* damage levels, water status, cane variety and cane age). The first derivatives from this region were used to determine the maximum red-edge inflexion point or REP. The movements of the REP slopes and the red-edge maxima were used to monitor Chlorophyll and N concentration as well as plant stress either caused by nutrient deficiency or *E. saccharina* damage or water content reduction. Normally under plant stress conditions, the REP slope and maximum red-edge inflexion point shift to the shorter wavelengths, due to low chlorophyll or N contents. This is called "blue-shift". But if there are high chlorophylls or N contents, they both shift to the longer wavelengths, resulting in the process called "red-shift".

3.5.2 Spectral Vegetation Indices

Spectral vegetation indices were considered to show how spectral slopes are sensitive to changes caused by *E. saccharina* in leaf biochemical properties (N and Si concentrations, N/Si) as well as water content of sugarcane crops. Therefore previously used narrow waveband spectral indices that are sensitive to leaf nutrients and pigments such as N and chlorophyll as well as those sensitive to water status were tested in this study (see Table 3.1). A correlation matrix was conducted between these spectral indices and all biochemical concentrations (N concentration, Si concentration, N/Si ratio, and water content) as well as *E. saccharina* stalk damage to determine which spectral indices can be used for estimation of these variables. Spectral indices which gave highest significant correlations were further used to develop regression models for estimation of these variables. The overall project as well as analyses undertaken is summarized in Figure 3.1.

Table 3.1 Spectral vegetation indices used in this study

Vegetation Index	Formula	Reference
Ratio Vegetation Index	RVI = (R810/R560)	Xue *et al*., 2004
Photochemical Reflectance Index	PRI = (R531-R570)/(R531+R570)	Sims & Gamon, 2002
Plant Senescence Reflectance Index	PSRI = (R680-R500)/R750	Sims & Gamon, 2002
Modified Spectral Ratio	mSR = (R750-R445)/(R705-R445)	Sims & Gamon, 2002
Red-Edge Index	REI = R740/R720	Vogelmann *et al*., 1993
Carter Index	CI = R760/R695	Cater, 1994
Noramalized Pigment Chlorophyll Index	NPCI = (R680-R430)/(R680+R430)	Penuelas *et al.*, 1994
Gitelson & Merzylak Index	GMI = R750/R700	Gitelson & Merzlyak, 1997;Ferri *et al.*, 2004
Normalized Difference	ND = (R1075-R730)/(R1075+R730)	Zhao *et al*., 2005
Normalized Difference Nitrogen Reflectance Index	NDNRI = (R1770-R693)/(R1770+R693)	Ferwerda *et al.,* 2005
Normalized Difference Vegetation Index	NDVI = (R750-R560)/(R750+R560)	Ferri *et al.*, 2004; Xue *et al*., 2004
Modified NDVI	mNDVI = (R2200-R2025)/(R2200+2025)	Abdel-Rahman *et al.*, 2008a
Spectral Ratio (Derivatives)	SR = D744/D2142	Abdel-Rahman *et al.*, 2008a
Water Band Index	WBI = R970/R900	Ray *et al*., 2006
Water Band Ratio	WBR = R960/R930	Ray *et al*., 2006
First derivatives at 730, 740, 744	D730, D740, D744	Zhao *et al*., 2005; Abdel-Rahman *et al.*, 2008a

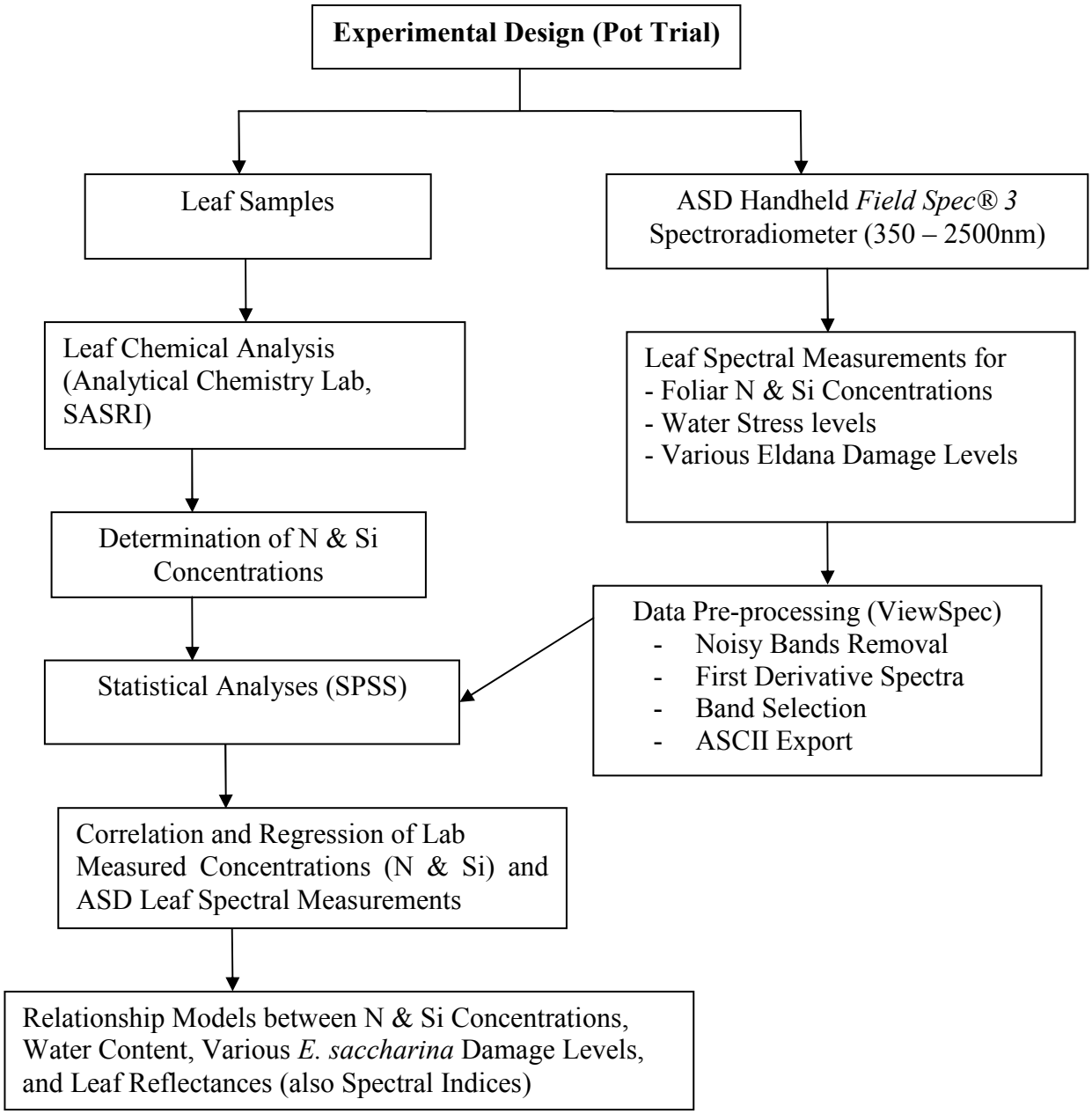


Fig. 3.1 Flow diagram illustrating the overall project design and process flow.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents the results and findings of this study as well as their analyses and interpretation. It further goes into discussions of these results and relates them with past studies as indicated in the literature review.

4.2 Research Findings

4.2.1 Treatments and Variety Effects on Foliar Biochemical Concentrations and *E. saccharina* Stalk Damage

A Factorial ANOVA was performed to determine if different factors (N treatments, Si treatments and variety) and their interactions had significant effects on foliar biochemical concentrations (N concentration, Si concentration, N/Si ratio as well as water content) throughout different cane ages (7, 10 and 12 months old cane) as well as on *E. saccharina* stalk damage on 12 months old cane.

For 7 months old cane, a Factorial ANOVA (see Appendix B) was performed with the purpose of determining whether there were significant differences caused by the three factors (variety, N treatment and Si treatment) as well as their interactions on the foliar biochemical concentrations (N concentration, Si concentration, and N/Si ratio). The results indicate that N treatment and variety are the two factors that had significant effects on foliar N concentration with $p < 0.05$ and $p < 0.001$, respectively. Silicon concentration on the other hand was significantly influenced by Si treatment and the interaction effect between N and Si treatments (N*Si treatments) with $p < 0.001$ and $p < 0.05$, respectively. N/Si ratio was significantly affected by Si treatment only, $p < 0.05$. All the interactions did not seem to influence the dependent variables except for N*Si treatment which significantly influenced Si concentration.

A Factorial ANOVA was also performed on 10 months old cane to determine whether there were significant effects brought by all factors (variety, N treatment and Si treatment) as well

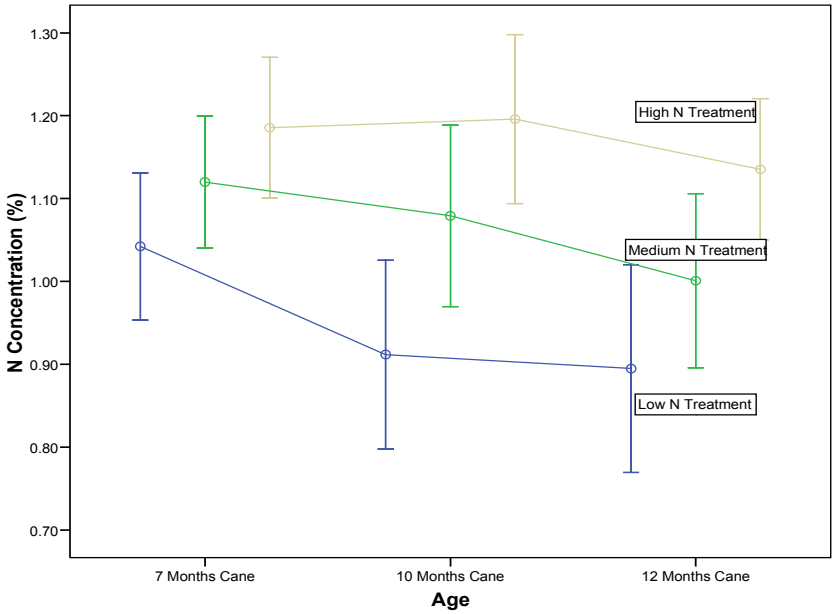
as their interactions on the foliar biochemical concentrations (N concentration, Si concentration, N/Si ratio and water content) (see Appendix C). The results show that only N treatment had significant influence on foliar N concentration ($p < 0.01$) while Si treatment and variety statistically affected foliar Si concentration with $p < 0.01$ and $p < 0.05$, respectively. None of the independent variables had statistical effects on both N/Si ratio and water content.

A Factorial ANOVA was also performed for 12 months old cane to investigate significant effects of the factors (variety, N treatment and Si treatment) as well as their interactions on the dependent variables (N concentration, Si concentration, N/Si ratio, water content, and *E. saccharina* stalk damage). This is shown in Appendix D. The results demonstrate that foliar N concentration was statistically influenced by N treatment and variety, $p < 0.01$ for both factors while Si was highly significantly affected by Si treatment and variety with $p < 0.01$ as well. Both N and Si treatments had significant impacts on N/Si ratio ($p < 0.001$). Water content was significantly affected by variety, N treatment, and N*Si treatment, $p < 0.001$, $p < 0.05$ and $p < 0.01$, respectively. Stalk damage by *E. saccharina* borer was significantly influenced by variety and Si treatment with $p < 0.01$ for both factors. Generally, variety had highly significant effect on all dependent variables ($p < 0.01$) except on N/Si ratio where $p = 0.07$. Therefore, varietals consideration for further statistical analyses worth highlighting.

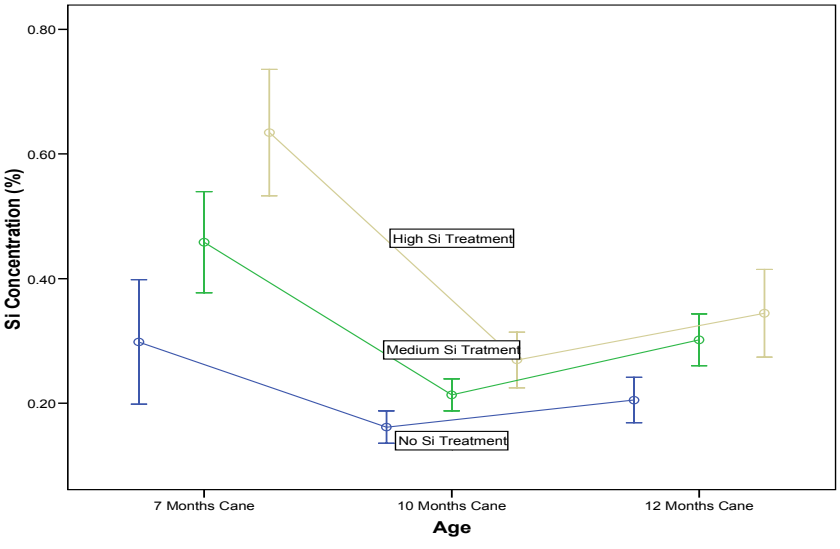
All the above results of Factorial ANOVAs made it realistic to try to look into the trend on the effects of N and Si treatments on foliar biochemical concentrations within different cane ages (Figure 4.1). Figure 4.1a highlights that foliar N concentration is increasing with increase in N treatment at all ages. However, there was a non-significant decrease in foliar N concentrations for almost all N treatments from 10 months to 12 months age. There are two possible reasons for this decrease, that is N application was stopped and water stress began at 9 months age and also the trial was artificially inoculated with *E. saccharina* eggs immediately after leaf samples for 10 months cane had been taken.

There was also increasing trend in foliar Si concentration with Si treatments in all ages (Figure 4.1b). There was a sharp decrease in foliar Si concentration for all Si treatments from 7 months cane to 10 months cane, ideally the sharp decrease was at least expected on N concentration as it was after termination of N application. The reasons for the sharp decrease in foliar Si concentration might be that irrigation water which could be potential source of Si was reduced, and also the trial was transferred to shade house therefore the contribution of

rain water which has Si ceased, even though rain Si is not of agronomic importance (Savant *et al.,* 1999). This is supported by the fact that sugarcane is a Si accumulator crop hence it responds vigorously to Si supply (Savant *et al.,* 1999). After 10 months the cane started to recover in leaf Si accumulation as there was a slight increase in foliar Si concentration for 12 months old cane.



(a)



(b)

Fig. 4.1 Relationships between foliar concentrations ((a) N, (b) Si) and N & Si treatments at different ages (7 months, 10 months and 12 months). For N treatments, low N (30 ppm), medium N (60 ppm) and high N (90 ppm) while for Si treatments, no Si (0 ppm), medium Si (100 ppm) and high Si (200 ppm). Error bars are Standard Errors (SE).

Figure 4.2 shows accumulation of biochemical concentrations (N concentration, Si concentration and N/Si ratio) within each variety as affected by age. The results show slightly

decreasing trend in foliar N concentration with increasing age in N25 and N37, with N14 being exceptional (Figure 4.2a). This was expected as leaf chemical analysis for 10 months old cane was done a month after N application was stopped. For 12 months cane, there are two possible reasons for reduction in N concentration, the first being stopping N application at 9 months. The second being the effect of stress (caused by water stress and *E. saccharina* damage) as plant chlorophyll and N concentrations tend to decrease more rapidly under stress conditions or during senescence (Sims and Gamon, 2002).

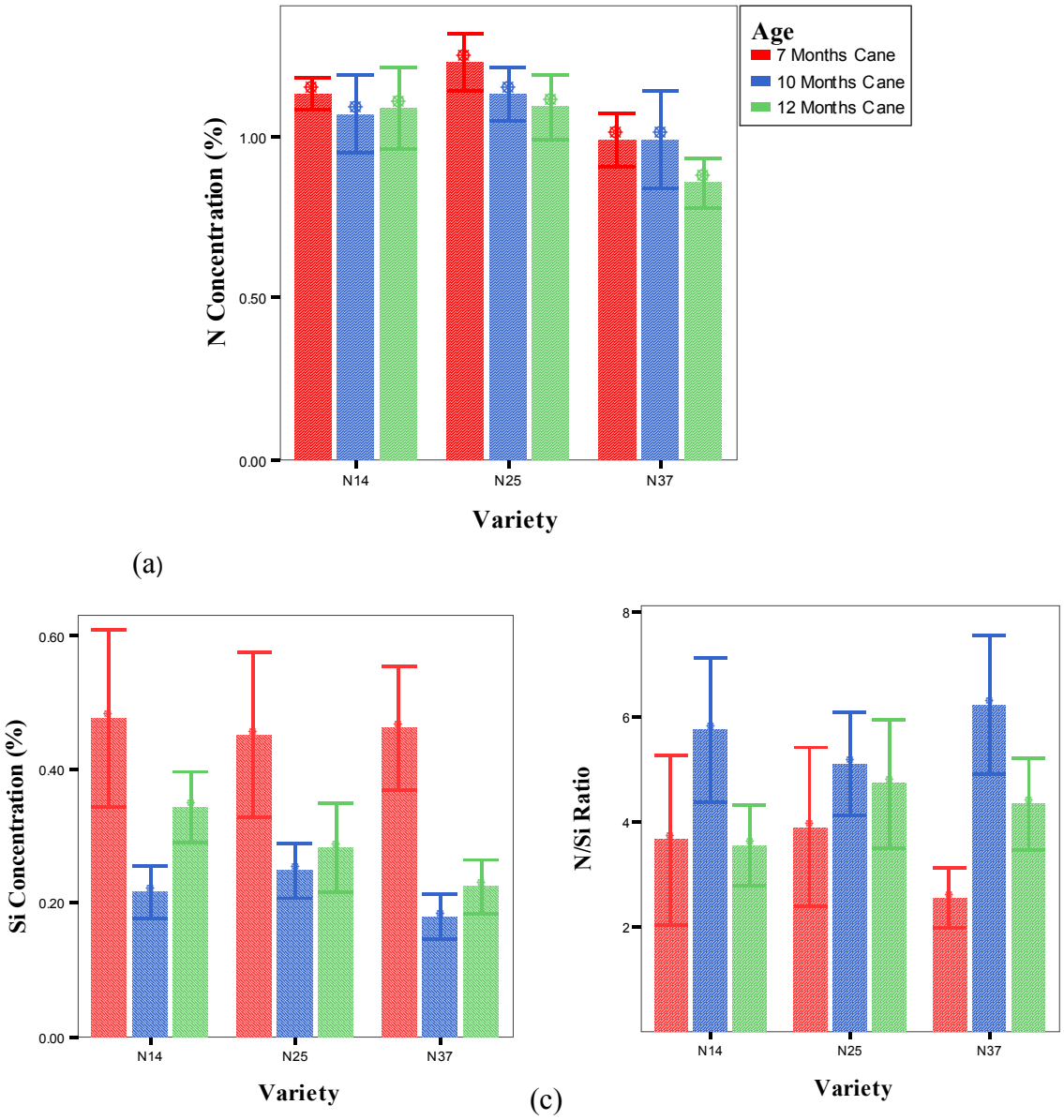


Fig. 4.2 Foliar concentrations ((a) N, (b) Si & (c) N/Si Ratio) in three varieties (N14, N25 &N37) at different cane ages (7 months, 10 months & 12 months). Error bars are SE.

Figure 4.2a also highlights that N37, which is intermediate-susceptible to *E. saccharina,* had lowest N concentrations in all three ages compared to the other two varieties. This implies that *E. saccharina* damage on the stalk reduces leaf N concentration, however this is still open for a follow-on study for validation. Silicon concentration was significantly higher for 7 months old cane in all varieties, then dropped for 10 months old cane and increased slightly for 12 months old cane (Figure 4.2b). This indicates a similar pattern with Figure 4.1b. N37 also had the lowest Si concentration on 12 months old cane, hence it was the most damaged variety by *E. saccharina* pest. N/Si ratio was lowest in 7 months old cane due to high Si concentrations in this cane age (Figure 4.2c). For N14, susceptible to *E. saccharina,* there are higher foliar Si concentrations than on the other two (N37 and N25) varieties on 12 months old cane (Figure 4.2b).

4.2.1.1 The Effects of N and Si Treatments on Reaction of Sugarcane Varieties to *E. saccharina* Stalk Damage

As it was stated earlier that stalk damage by *E. saccharina* borer was significantly influenced by variety, $p < 0.01$ (Appendix D), a further step was taken to investigate if N and Si treatments had significant effects on reaction of sugarcane varieties to *E. saccharina* stalk damage by performing a Factorial ANOVA (Table 4.1). See also Figure 4.3.

Table 4.1 Results of Factorial ANOVA illustrating significant differences caused by N and Si treatments as well as their interaction on stalk damage by *E. saccharina* within varieties.

Variety	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N14	Corrected Model	6519.444(a)	8	814.931	0.855	0.582
	Intercept	37355.556	1	37355.556	39.207	0.00001
	N treatment	86.111	2	43.056	0.045	0.956
	Si treatment	5619.444	2	2809.722	2.949	0.104
	N treatment * Si treatment	813.889	4	203.472	0.214	0.924
N25	Corrected Model	9412.778(b)	8	1176.597	3.778	0.032
	Intercept	22190.222	1	22190.222	71.249	0.00001
	N treatment	2403.444	2	1201.722	3.859	0.062
	Si treatment	6350.111	2	3175.056	10.195	0.005
	N treatment * Si treatment	659.222	4	164.806	0.529	0.718
N37	Corrected Model	6177.778(c)	8	772.222	1.979	0.165
	Intercept	84734.722	1	84734.722	217.114	0.00001
	N treatment	1969.444	2	984.722	2.523	0.135
	Si treatment	1002.778	2	501.389	1.285	0.323
	N treatment * Si treatment	3205.556	4	801.389	2.053	0.17

Table 4.1 indicates that N treatment does not have an influence on *E. saccharina* stalk damage for any variety (also see Figure 4.3a). Figure 4.3b shows that Si treatment increased resistance to damage by *E. saccharina* in N14 and N25. However, the only significant effect of Si treatment on *E. saccharina* stalk damage was on N25 ($p < 0.01$, Table 4.1), hence N25 was the least damaged variety by *E. saccharina.* It is worth to highlight that N37, which is intermediate-susceptible to *E. saccharina*, was the most damaged compared to the other two varieties (N14, most susceptible and N25, intermediate-susceptible) under different N and Si treatments (Figure 4.3). Although N14 was the most susceptible variety, it was not the most damaged by *E. saccharina* due to its strong uptake of Si nutrient which resulted in high its higher foliar Si concentration on 12 months old cane (Figure 4.2b).

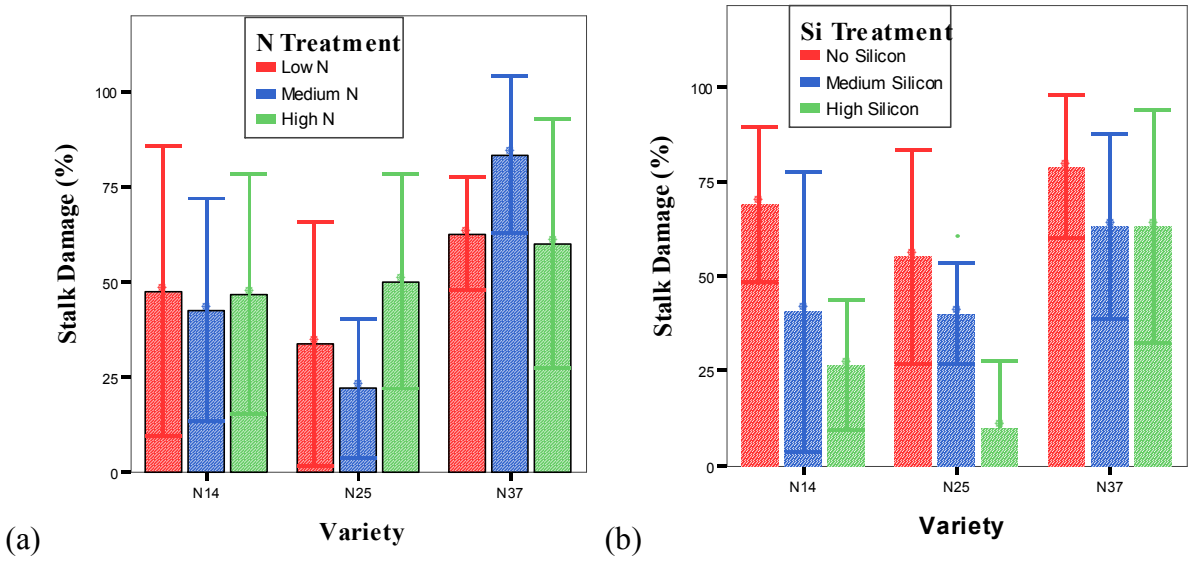


Fig. 4.3 Percentage stalk damage at age 12 months in three varieties (N14, N25 & N37) under different treatments. (a) N treamnets and (b) Si treatments. Error bars are SE.

4.2.1.2 Correlation Between *E. saccharina* Stalk Damage and All Variables

Pearson's correlation was performed with the aim of determining the relationship between *E. saccharina* stalk damage against all variables (both independent and dependent) on 12 months old cane (see Table 4.2). The highest significant correlation coefficient was from the relationship of *E. saccharina* stalk damage and number of *E. saccharina* larvae and pupae recovered from cane stalks ($r = 0.78$; $p < 0.05$) (see Table 4.2).

Table 4.2 Pearson's correlation matrix of all variables (both independent and dependent) for 12 months old cane for all varieties combined.

	N Conc. (%)	Si Conc. (%)	N/Si Ratio	AWC (%)	Number of Eldana	Stalk Damage (%)	N Treatment	Si Treatment
N Conc. (%)	1	0.007	.421(**)	.380(**)	-0.01	-0.071	.427(**)	-0.032
Si Conc. (%)	0.007	1	-.779(**)	0.143	-0.2	-.456(**)	-0.261	.488(**)
N/Si Ratio	.421(**)	-.779(**)	1	0.097	0.161	.314(*)	.493(**)	-.429(**)
AWC (%)	.380(**)	0.143	0.097	1	-0.228	-0.164	0.149	0.154
Number of Eldana	-0.01	-0.2	0.161	-0.228	1	.784(**)	0.136	-.297(*)
Stalk Damage (%)	-0.071	-.456(**)	.314(*)	-0.164	.784(**)	1	0.06	-.475(**)
N Treatment	.427(**)	-0.261	.493(**)	0.149	0.136	0.06	1	0
Si Treatment	-0.032	.488(**)	-.429(**)	0.154	-.297(*)	-.475(**)	0	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
Number of Eldana = Total Number of Larvae and Pupae

Table 4.2 also illustrates that both Si treatment and leaf Si concentration were significantly correlated *E. saccharina* stalk damage ($r = -0.48$; $p < 0.01$; and $r = -0.46$; $p < 0.01$, respectively). Negative correlation coefficients indicate that Si treatment and leaf Si concentration were inversely proportional to *E. saccharina* stalk damage. This confirms the finding by other studies, indicated in the literature review (Savant *et al.,* 1999; Kvedaras *et al.*, 2007), as negative correlations proved that Si aids in the resistance of cane plants to infestation by *E. saccharina*, which later reduces the degree of sugarcane stalk damage caused by *E. saccharina.* N/Si ratio was slightly correlated to *E. saccharina* stalk damage ($r = 0.31$; $p < 0.05$) while variety also had a highly significant relationship with *E. saccharina* stalk damage ($r = 0.32$; $p < 0.05$).

This makes it worthwhile to perform the same Pearson's correlation matrix at different varieties (N14, N25 and N37). This is highlighted in Table 4.3. The results from Table 4.3 indicate that the relationship between *E. saccharina* stalk damage and Si (Si treatment ($r = -0.60$; $p < 0.01$) and Si concentration ($r = -0.44$; $p > 0.05$)) increased slightly for N14 while a high increase was observed for N25, that is *E. saccharina* stalk damage and Si (Si treatment and Si concentration) with $r = -0.71$; $p < 0.01$ and $r = -0.49$; $p < 0.05$, respectively. This further supports the earlier statement that Si treatment increased resistance to damage by *E. saccharina*, by impeding larval penetration into stalks (Kvedaras and Keeping, 2007), in all varieties but more especially in N14 and N25 (Figure 4.3b). For N37, there was poor non-significant relationship between *E. saccharina* stalk damage and Si (Si treatment and Si

concentration, r = -0.28; p > 0.05 and r = -0.23; p > 0.05, respectively) (Table 4.3). This indicates that Si content did not help in *E. saccharina* stalk damage reduction in this variety hence N37 was the most damaged by *E. saccharina.*

N/Si ratio was only significantly related to *E. saccharina* damage on N25 (r = 0.52; p < 0.05). Surprisingly, water was not related to *E. saccharina* damage at any variety even though r = 0.33; p > 0.05 on N25 which is higher than when varieties combined together (r = -0.16; p > 0.05) (Table 4.3).

Table 4.3 Correlations matrix of all cane variables (both dependent and independent) within varieties.

Variety		N Conc. (%)	Si Conc. (%)	N/Si Ratio	AWC (%)	Number of Eldana	Stalk Damage (%)	N Treatment	Si Treatment
N14	N Concentration (%)	1	-0.248	.652(**)	0.114	0.144	0.132	.516(*)	-0.161
	Si Concentration (%)	-0.248	1	-.841(**)	-0.07	-0.061	-0.439	-0.132	.549(*)
	N/Si Ratio	.652(**)	-.841(**)	1	0.234	0.052	0.354	0.412	-0.468
	AWC (%)	0.114	-0.07	0.234	1	-0.063	0.111	0.201	0.107
	Number of Eldana	0.144	-0.061	0.052	-0.063	1	.755(**)	0.196	-0.458
	Stalk Damage (%)	0.132	-0.439	0.354	0.111	.755(**)	1	-0.012	-.599(**)
	N Treatment	.516(*)	-0.132	0.412	0.201	0.196	-0.012	1	0
	Si Treatment	-0.161	.549(*)	-0.468	0.107	-0.458	-.599(**)	0	1
N25	N Concentration (%)	1	-0.197	.522(*)	0.404	.546(*)	0.416	0.289	-0.101
	Si Concentration (%)	-0.197	1	-.784(**)	-0.095	-0.313	-.485(*)	-0.33	0.467
	N/Si Ratio	.522(*)	-.784(**)	1	0.282	.570(*)	.521(*)	.512(*)	-0.355
	AWC (%)	0.404	-0.095	0.282	1	0.325	0.329	0.248	-0.068
	Number of Eldana	.546(*)	-0.313	.570(*)	0.325	1	.756(**)	0.328	-0.417
	Stalk Damage (%)	0.416	-.485(*)	.521(*)	0.329	.756(**)	1	0.259	-.708(**)
	N Treatment	0.289	-0.33	.512(*)	0.248	0.328	0.259	1	0
	Si Treatment	-0.101	0.467	-0.355	-0.068	-0.417	-.708(**)	0	1
N37	N Concentration (%)	1	-0.141	0.451	0.264	0.079	-0.043	.731(**)	0.254
	Si Concentration (%)	-0.141	1	-.854(**)	0.453	-0.204	-0.231	-0.438	.678(**)
	N/Si Ratio	0.451	-.854(**)	1	-0.352	0.228	0.239	.623(**)	-.574(*)
	AWC (%)	0.264	0.453	-0.352	1	-0.103	-0.116	0.092	.496(*)
	Number of Eldana	0.079	-0.204	0.228	-0.103	1	.826(**)	0.029	-0.172
	Stalk Damage (%)	-0.043	-0.231	0.239	-0.116	.826(**)	1	-0.044	-0.279
	N Treatment	.731(**)	-0.438	.623(**)	0.092	0.029	-0.044	1	0
	Si Treatment	0.254	.678(**)	-.574(*)	.496(*)	-0.172	-0.279	0	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed

Number of Eldana = Total Number of Larvae and Pupae

4.2.2 Leaf Reflectance

4.2.2.1 Differences Between Leaf Reflectance under Different N and Si Treatments at All Varieties Combined

One-way ANOVA was conducted to investigate which wavebands of the visible-NIR portion spectrum ranges (400 – 1300 nm) can significantly distinguish N and Si treatments on different cane ages for all combined varieties (Figure 4.4 and 4.5, respectively). Figure 4.4a highlights that spectral reflectance of cane plants treated with three levels of N differed at 510 – 640 nm and 690 – 740 nm bands on 7 months cane. These bands have been previously found to be sensitive to chlorophyll and N contents (Kumar *et al.,* 2003; Zhao *et al.,* 2005). Nitrogen treatment also had statistical influence on reflectance at 400 – 740 nm bands ($p < 0.05$) on 10 months cane (Figure 4.4b). However, none of the wavebands were able to significantly distinguish different N treatments on 12 months cane (Figure 4.4c). This is due to the effects of stopping N application and beginning of water stress at age of 9 months, as well as effects of *E. saccharina* stalk damage as the plants were artificially inoculated with *E. saccharina* eggs a month after the beginning of water stress.

Figure 4.5a indicates that there were highly significant differences from 400 - 740 nm, thus wavebands within the visible and part of the red-edge, the shorter wavelengths, are the most bands that can pick the significant differences between Si treatments ($p < 0.01$) on 7 months cane. This confirms that higher leaf reflectance in the shorter wavelengths are due to higher contents of silicates in the leaves (Alvarez-Añorve *et al.,* 2008). In contrary, for 10 months cane, the longer wavelengths (NIR region), from 740 - 930 nm and 960 - 1060 nm, were the ones which were able to statistically distinguish between different Si treatments with $p < 0.05$ (Figure 4.5b). This is related to the sharp decrease in Si concentration on the 10 months old cane as explained earlier (Figures 4.1b and 4.2b). For 12 months cane, Si treatments did not have significant effects on reflectance at any wavebands (Figure 4.5c). This might be due to the effects of water stress as plants were subjected to water stress from the age of 9 months and effects of *E. saccharina* stalk damage as plants were artificially inoculated with *E. saccharina* eggs a month after the beginning of water stress.

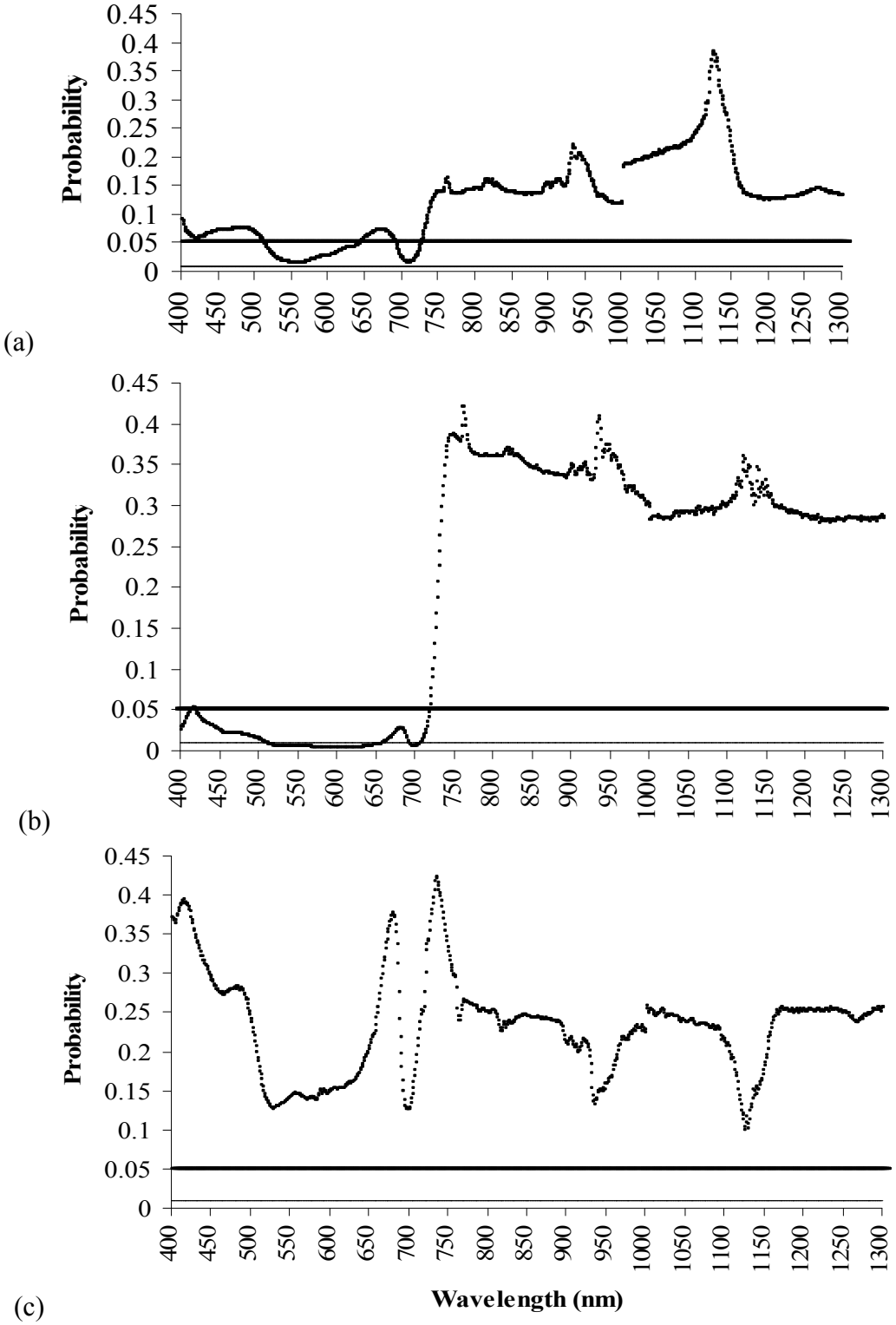


Fig. 4.4 Results of one-way ANOVA illustrating wavebands of the visible-NIR portion spectrum ranges (400 – 1300 nm) that can significantly distinguish N treatments on different cane ages. (a) 7 months, (b) 10 months and (c) 12 months. Thinner and thicker horizontal lines indicate 0.01 and 0.05 significance levels (99 % and 95 % confidence limits), respectively.

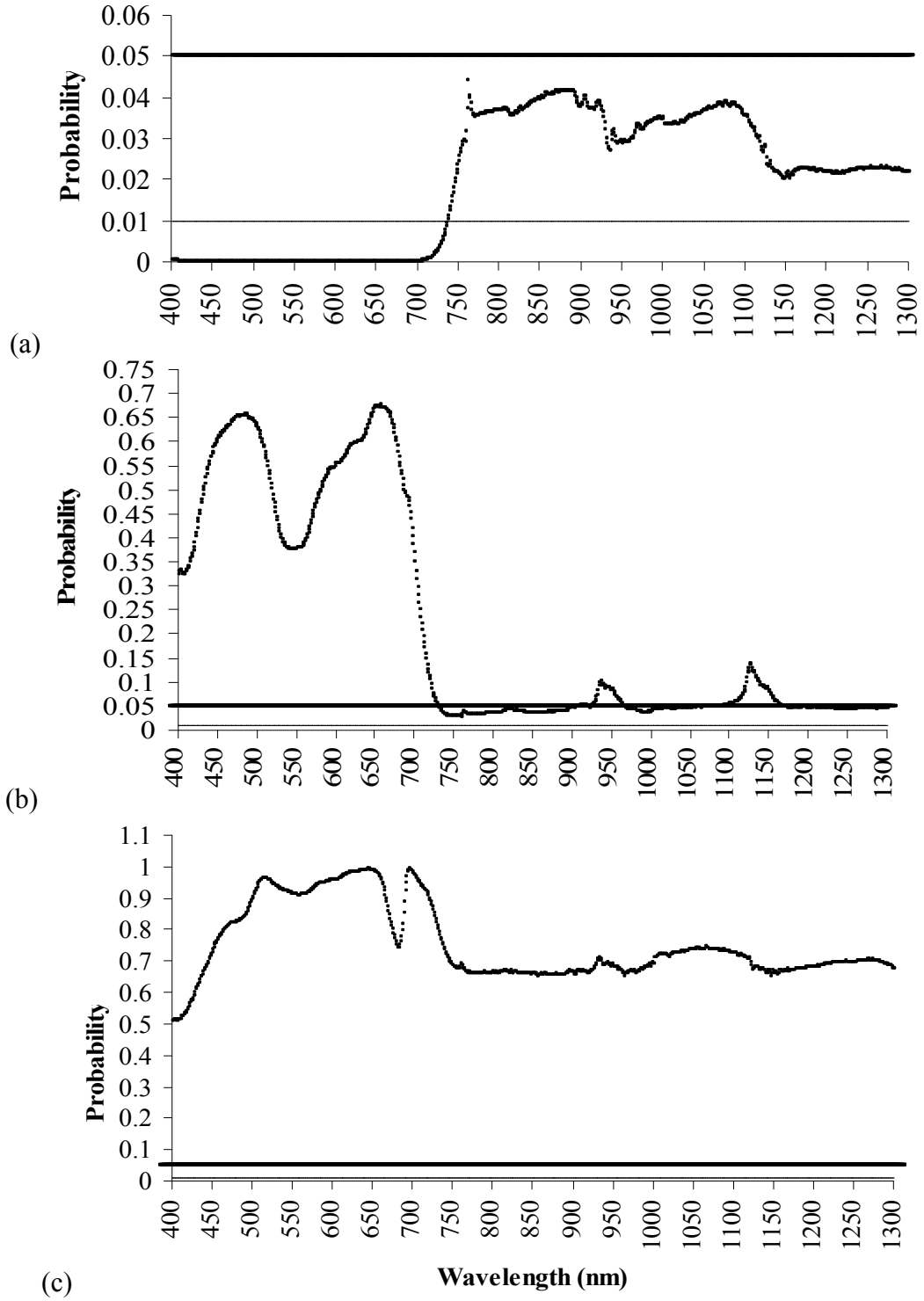


Fig. 4.5 Results of one-way ANOVA illustrating wavebands of the visible-NIR portion spectrum ranges (400 – 1300 nm) that can significantly distinguish Si treatments on different cane ages. (a) 7 months, (b) 10 months and (c) 12 months. Thinner and thicker horizontal lines indicate 0.01 and 0.05 significance levels (99 % and 95 % confidence limits), respectively.

4.2.2.2 Discrimination Between Healthy and *E. saccharina*-infected Cane using Leaf Reflectance at All Varieties Combined

In order to discriminate between healthy and *E. saccharina* damaged cane using leaf reflectance, an ANOVA was performed as described in section 3.4.2 (Figure 4.6). Figure 4.6a indicates that there were highly significant differences in leaf reflectance from healthy and *E. saccharina* damaged cane throughout the spectrum ($P < 0.001$) (Table 4.4).

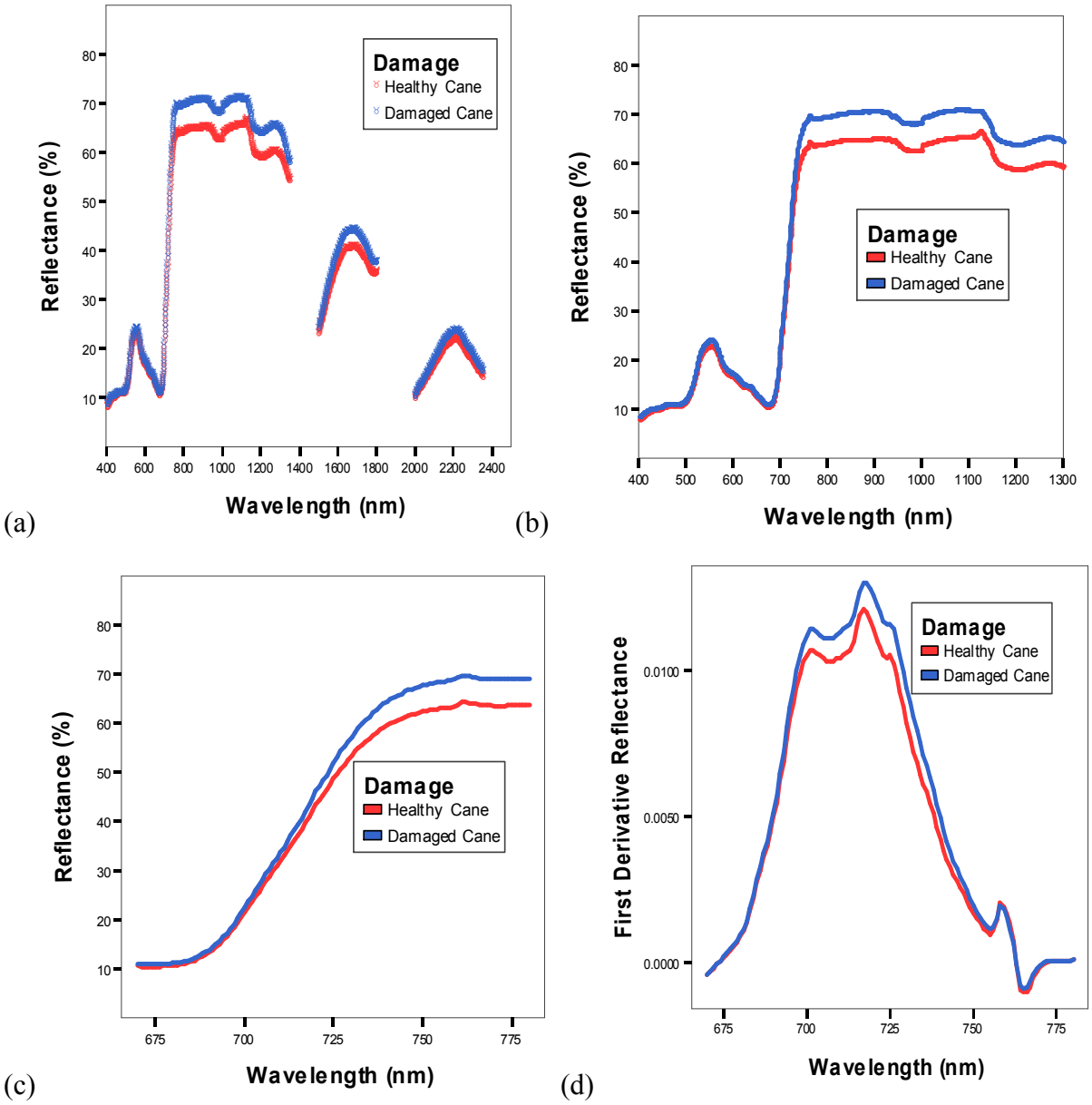


Fig 4.6 Mean leaf spectral reflectance curves highlighting healthy and E. saccharina damaged cane. (a) spectrum without noisy wavebands, (b) only visible-NIR portion of spectrum range (400 – 1300 nm), (c) and (d) Red-edge region.

Table 4.4 Results of ANOVA illustrating if wavebands from the whole spectrum as well as different portions of the spectrum can significantly discriminate between healthy and *E. saccharina* damaged cane.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	14.454	.00001
VNIR portion of spectrum range	10.064	.002
Red-edge (670 – 780 nm)	1.094	.297

The red-edge region was further assessed to investigate the effects of *E. saccharina* damage on leaf pigments and nutrients such as chlorophyll and N. Figure 4.6c shows that there was a shift of the red-edge slope towards shorter wavelengths for *E. saccharina* damaged cane. This is known as blue shift. This indicates that *E. saccharina* stalk damage caused a decrease in leaf chlorophyll and N concentrations as this shift is the result of low chlorophyll concentrations. However, this shift was not significant as $p > 0.05$, $p = 0.297$ (Table 4.4). Figure 4.6d highlights that both healthy and *E. saccharina* damaged cane had their maximum red-edge peaks around 720 nm with *E. saccharina* damaged cane having higher peaks.

4.2.2.3 Differences in Leaf Reflectance as influenced by *E. saccharina* Damage Levels for All Varieties Combined

In order to distinguish between various damage levels, the damage range (0 – 100%) was further categorized into four levels and then ANOVA was performed. The methodology or procedure was elaborated in detail in section 3.4.2. Figure 4.7 shows that there was a slight difference or variation in leaf reflectance at different wavebands as influenced by stalk damage levels throughout the spectrum.

Severely damaged sugarcane gave the highest reflectance, followed by medium damaged and then low damage and healthy plants were overlapping (Figure 4.7a). This further validates that stress (biotic or abiotic stress) increases reflectance from 1300 to 2500 nm as well as in the range of 400 to 1300 nm (Carter, 1991; 1993). An ANOVA was performed to test whether these damage levels showed significant differences on leaf reflectance spectra. The differences were highly significant throughout the spectrum range ($P < 0.001$) (Table 4.5).

Figure 4.7b clearly indicates that severely *E. saccharina*-damaged cane had the highest leaf reflectance followed by medium *E. saccharina*-damaged cane in the NIR region. This implies that *E. saccharina* damage on the cane stalks did not break down cell structures in the cane

leaves as a decrease in leaf reflectance in the NIR region is associated with breakdown of leaf cell structures (Datt *et al.,* 2006).

Figure 4.7c shows that there was slight (not significant, p = 0.41, see Table 4.5) effect of different *E. saccharina* stalk damage levels at the red-edge region of the spectrum, that is red-edge slopes of both severe and medium *E. saccharina* damage moved towards the shorter wavelengths indicating reduction in chlorophyll and N concentrations. These slight differences (Figure 4.7), are due to the effects of variety as different varieties have different spectral reflectance signatures (Apan *et al.,* 2004a; Galvão *et al.*, 2005). Therefore this made it worthwhile to consider *E. saccharina* stalk damage at each variety as it was indicated earlier that stalk damage was also different for different varieties (N37 more damaged than both N14 and N25). This is illustrated in Figures 4.8, 4.9, and 4.10.

Figures 4.7e-f show highly significant differences ($p < 0.0001$, Table 4.5) in leaf reflectance as influenced by various *E. saccharina* stalk damage levels in the SWIR (1500 – 1800 nm and 2000 – 2350 nm, respectively). Figure 4.7e shows that highest significant reflectance peaks for all damage levels were centred on 1660 nm, with severe damage having the highest reflectance. Figure 4.7f on the other side highlights that highest reflectance peaks were around 2200 nm, still with severe damage level having the highest reflectance, however there was no clear distinction between healthy and medium damage levels in this region.

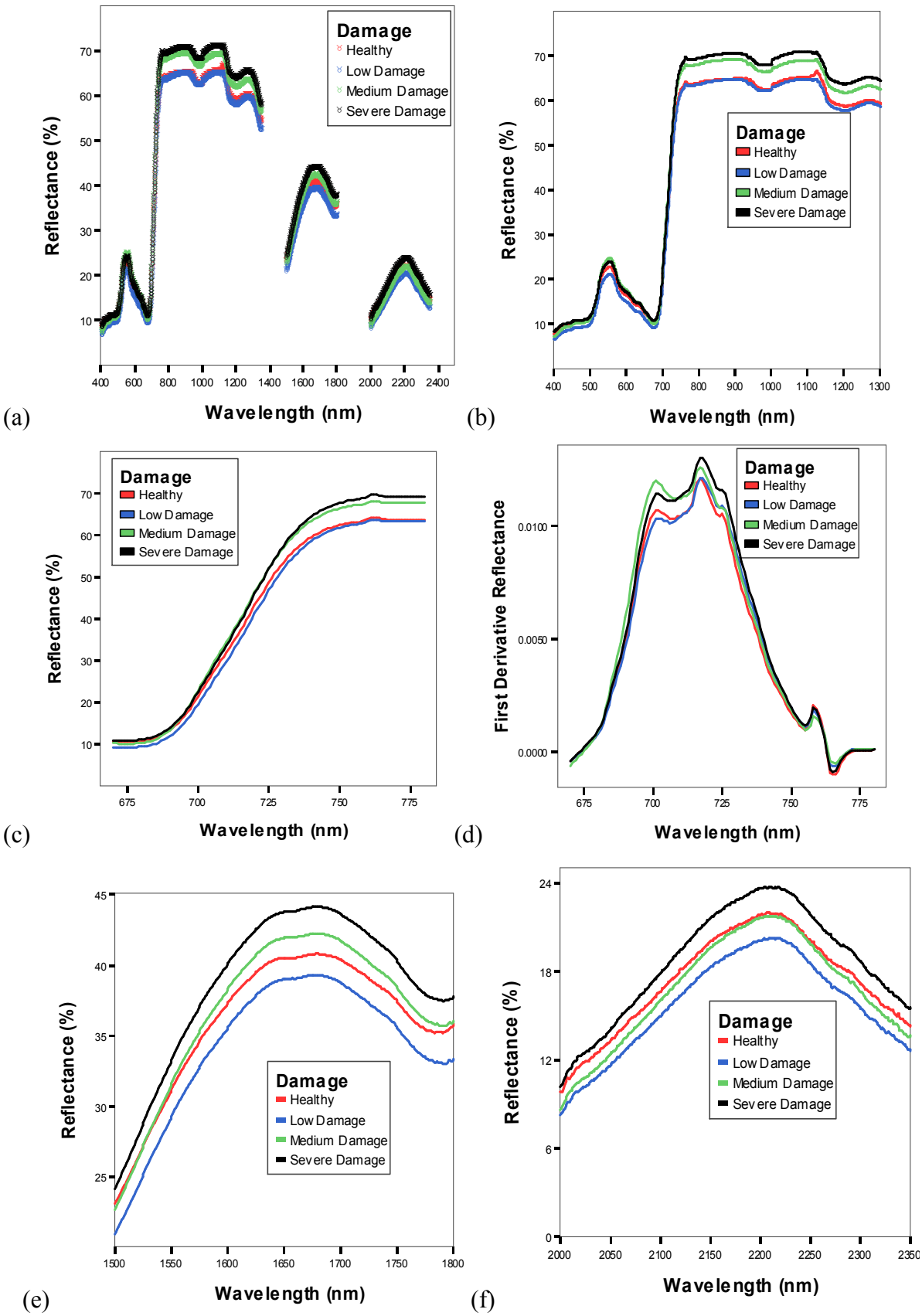


Fig 4.7 Leaf spectral reflectance curve showing different E. saccharina damage levels on 12 months cane. (Healthy – 0% stalk damaged, Low damage= 1 – 39% stalk damaged, Medium damage = 40 – 69% stalk damaged and Severe damage = 70 – 100% stalk damaged). (a) spectrum without noisy wavebands, (b) only visible-NIR portion of spectrum range (400 – 1300 nm), (c) and (d) Red-edge region, (e) 1500 - 1800 nm range and (f) 2000 – 2350 nm range.

Table 4.5 Results of ANOVA illustrating if wavebands from the whole spectrum as well as from different portions of the spectrum can statistically distinguish between various *E. saccharina* damage levels.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	11.056	.0001
Red-edge (670 – 780 nm)	.967	.408
(1500 – 1800 nm)	40.716	.0001
(2000 – 2350 nm)	45.379	.0001

4.2.2.4 Effects of *E. saccharina* Stalk Damage Levels on Leaf Reflectance for Different Varieties (N14, N25 and N37)

Figure 4.8 shows that there was difference in leaf reflectance of N14 caused by various damage levels by *E. saccharina* pest ($p = 0.019$ for whole spectrum and $p = 0.522$ for the red-edge region) however, severe damage level by *E. saccharina* reflected higher throughout the spectrum (Figures 4.8a, b and d; Table 4.6).

Figure 4.9 shows that, even though severely damaged cane had highest reflectance and healthy damage cane had the lowest reflectance throughout the spectrum, there was no significant difference in leaf reflectance on N25 as influenced by various damage levels by *E. saccharina* pest ($p = 0.071$ for whole spectrum and $p = 0.755$ for the red-edge region) (Table 4.7).

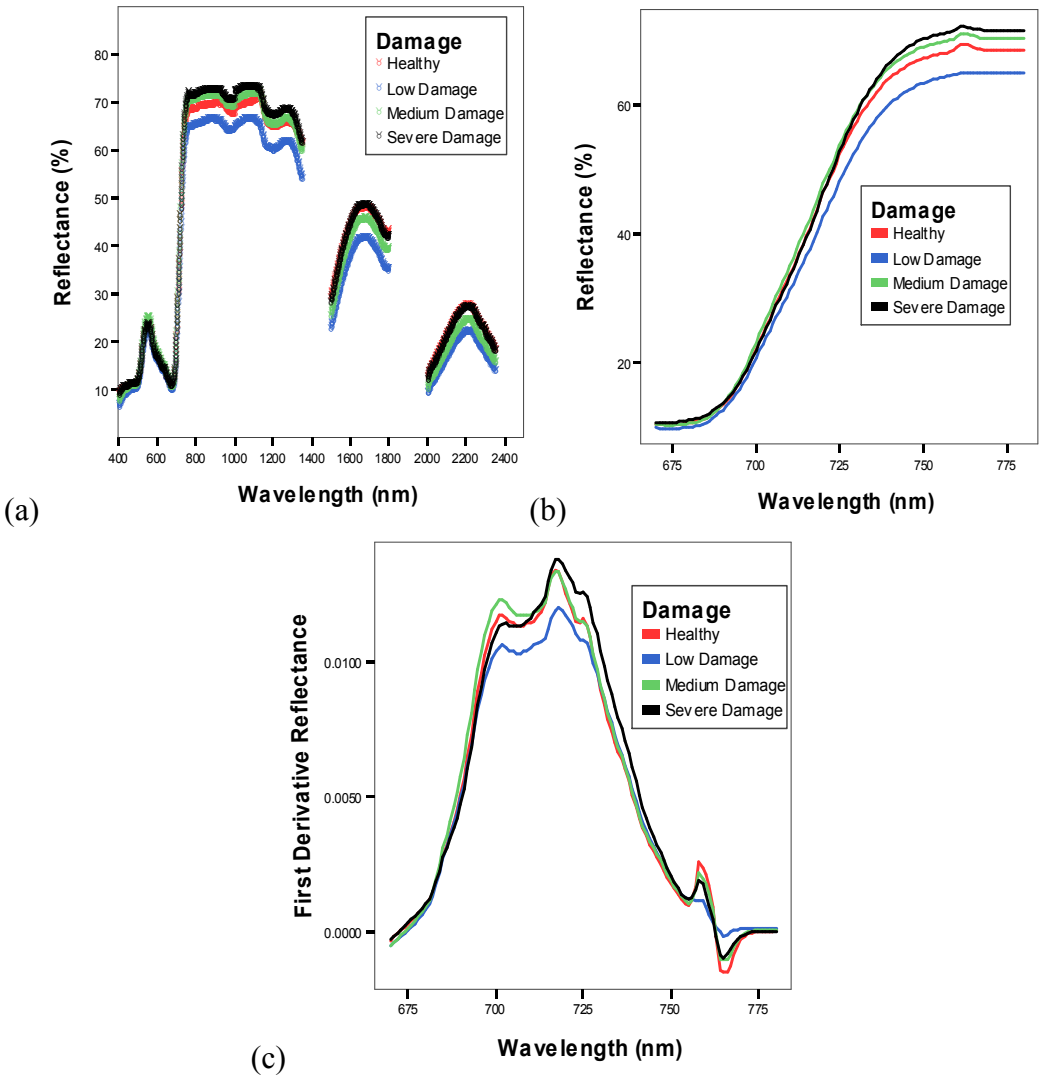


Fig 4.8 Leaf spectral reflectance curve showing different E. saccharina damage levels on 12 months N14 cane variety. (a) spectrum without noisy wavebands, (b) and (c) Red-edge region.

Table 4.6 Results of ANOVA highlighting whether leaf reflectance from the whole spectrum as well as from red-edge can statistically distinguish between various *E. saccharina* damage levels on N14.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	3.330	.019
Red-edge (670 – 780 nm)	.752	.522

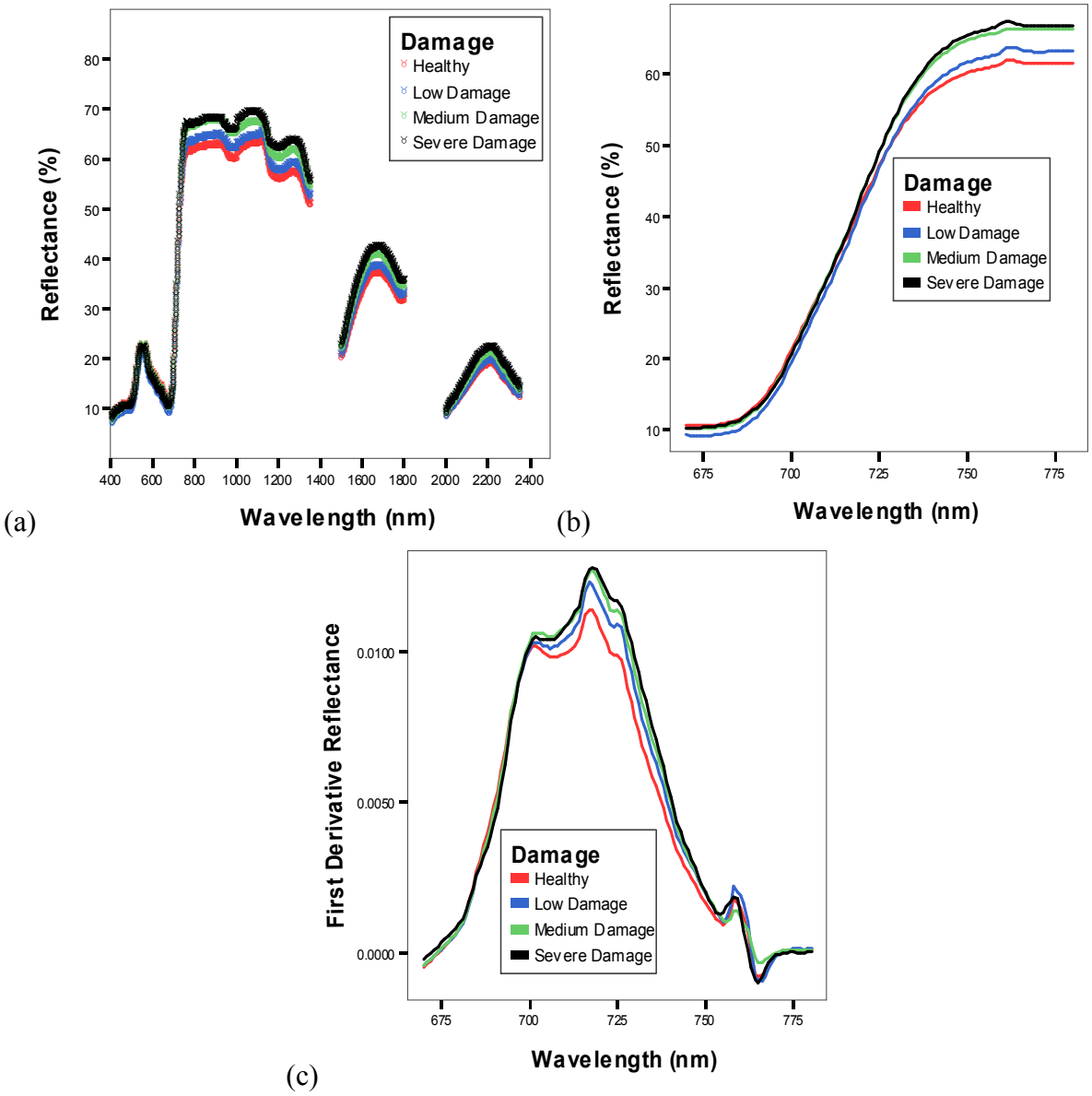


Fig. 4.9 Leaf spectral reflectance curve showing different E. saccharina damage levels on 12 months N25 cane variety (a) spectrum without noisy wavebands, (b) and (c) Red-edge region.

Table 4.7 Results of ANOVA illustrating if reflectance from the whole spectrum as well as from different portions of the spectrum can differentiate between various *E. saccharina* damage levels on N25.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	2.347	.071
Red-edge (670 – 780 nm)	.398	.755

Figure 4.10 shows that various *E. saccharina* damage levels had highly significant impacts on leaf spectral signature ($p < 0.0001$) of N37 cane variety (Table 4.8). Even though there was significant difference between all damage levels, it is interesting to note that there was no level for healthy cane in this variety, this indicates that all cane stalks from this cane variety were damaged by *E. saccharina* pest. It is also important to note the difference between severe damage level and medium damage level was small especially in the VNIR region (Figure 4.10a, b, and c). Figure 4.10b clearly illustrates that both severe and medium damage levels increased reflectance in the visible region which confirms that there was reduction in leaf pigments caused by *E. saccharina* damage on cane plant as this region is characterized by leaf pigment and nutrient absorption peaks (carotenoids, chlorophylls and N). This confirms that any physiological stress, pest and disease or reduced amount of photosynthesis increases red and blue reflectance (Nilsson, 1995).

In addition, both severe and medium damage levels red-edge slopes and REP significantly ($p \leq 0.01$, Table 4.8) shifted towards the shorter wavelengths indicating that *E. saccharina* damage highly induced both chlorophyll and N concentrations, causing the blue shift (Figure 4.10c and 4.10d). Figure 4.10d also highlights that both severe damage and low damage levels had REP maxima near 720 nm while medium damage had REP maximum at around 700 nm.

This implies that red-edge reflectance as well as its first derivative reflectance can be used to successfully assess and monitor various damage levels caused by *E. saccharina* in N37 cane variety. This is the only case whereby red-edge region had a significant difference ($p = 0.012$, Table 4.8) since the red-edge was assessed for different *E. saccharina* damage levels as well as when discriminating between healthy and *E. saccharina* damaged cane. Therefore, further an ANOVA was performed on N37 to look into which wavebands could be best used to discriminate different damage levels (Figure 4.11).

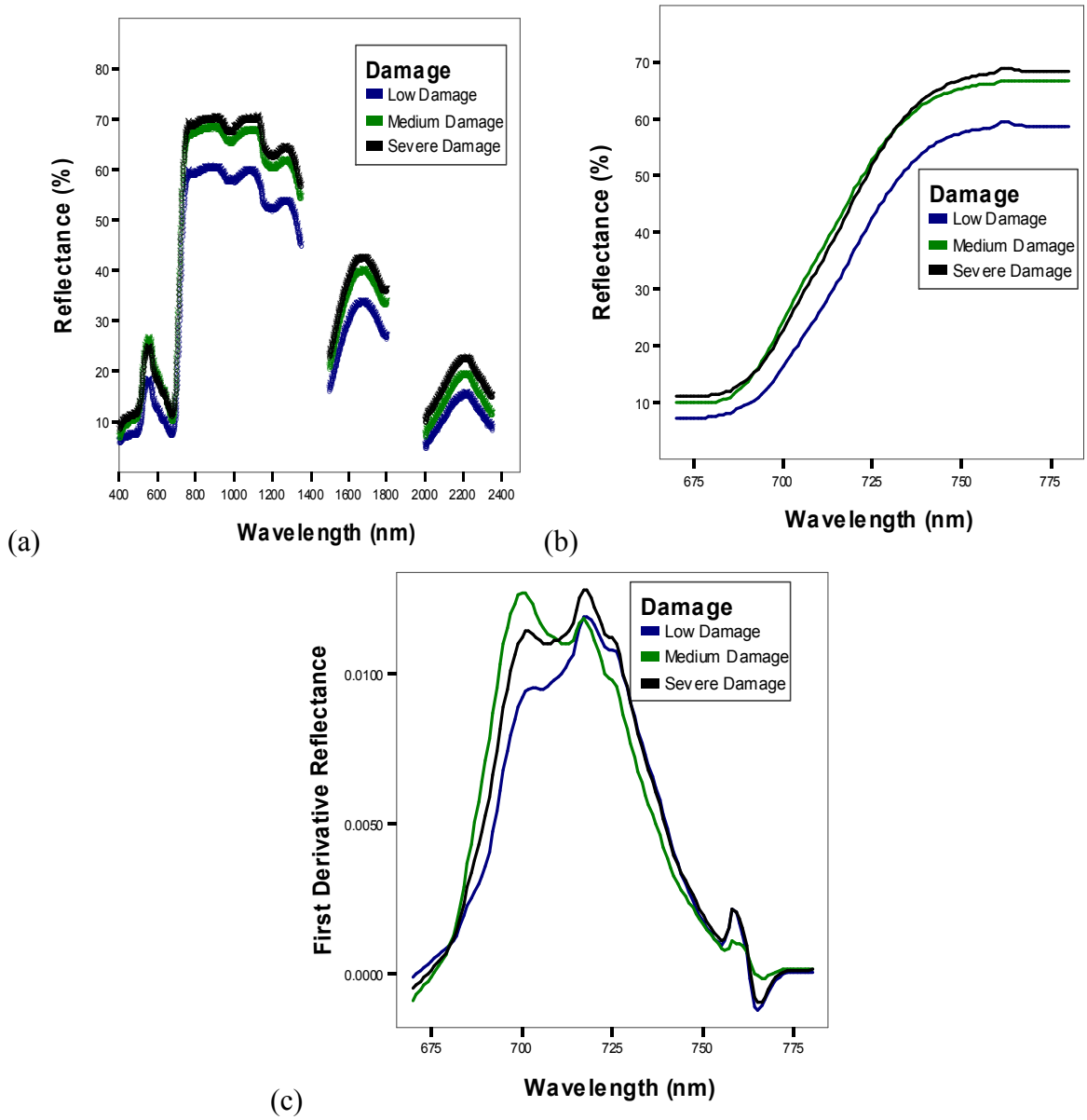


Fig. 4.10 Leaf spectral reflectance curve showing different E. saccharina damage levels on 12 months N37 cane variety (a) spectrum without noisy wavebands, (b) and (c) Red-edge region.

Table 4.8 Results of ANOVA indicating if leaf reflectance from the whole spectrum as well as from different portions of the spectrum can significantly distinguish between various *E. saccharina* damage levels of N37.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	29.713	.000
Red-edge (670 – 780 nm)	4.526	.012

Figure 4.11a shows that wavebands 410 – 430 nm and 2010 – 2340 nm could significantly distinguish between the *E. saccharina* damage levels ($p \leq 0.05$). The significance of bands 2010 – 2340 nm further validates the higher correlations between leaf reflectance and *E. saccharina* stalk damage which ranged from r = 0.5 – 0.6 in bands 2000 – 2350 nm (Figure 4.14).

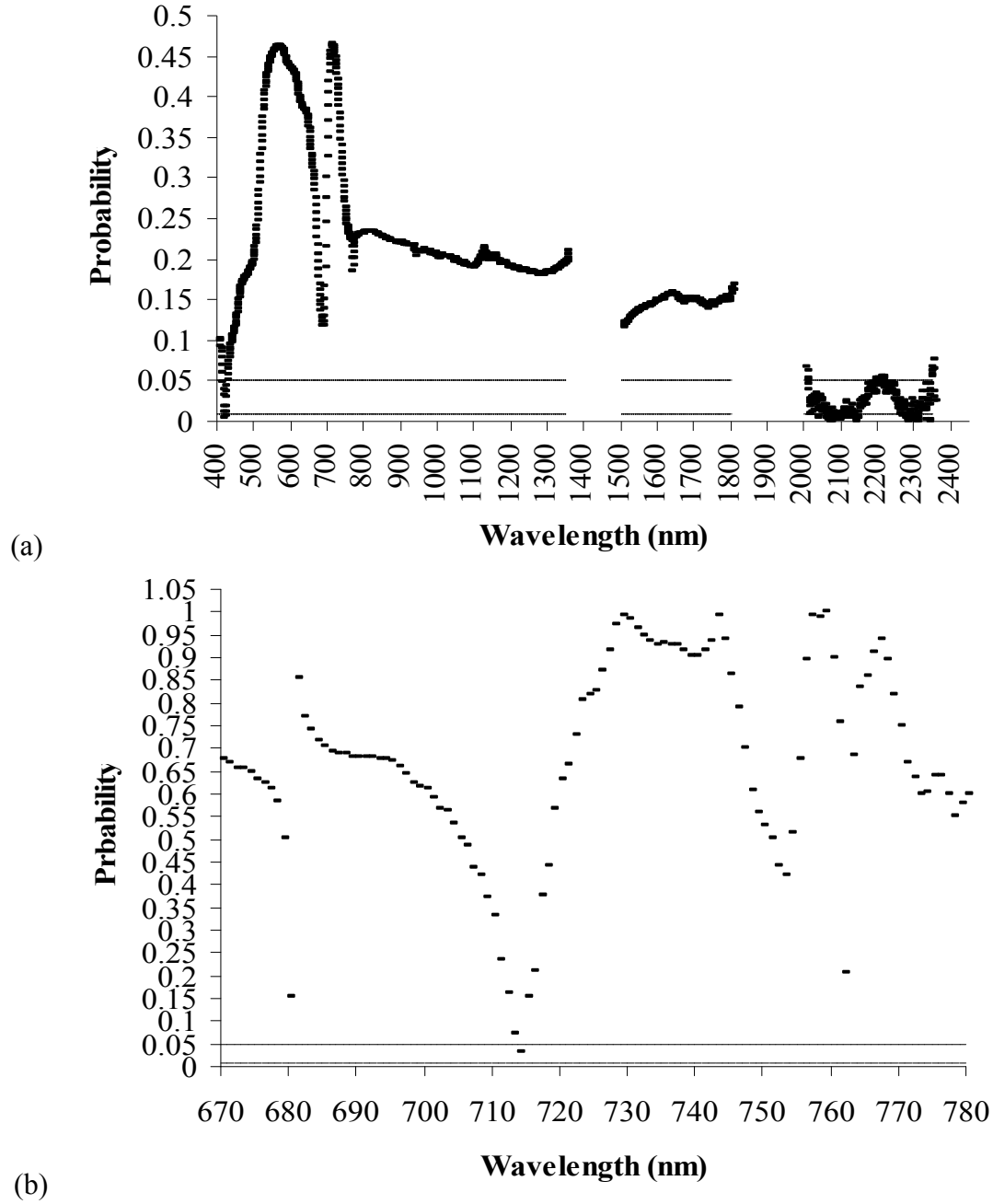


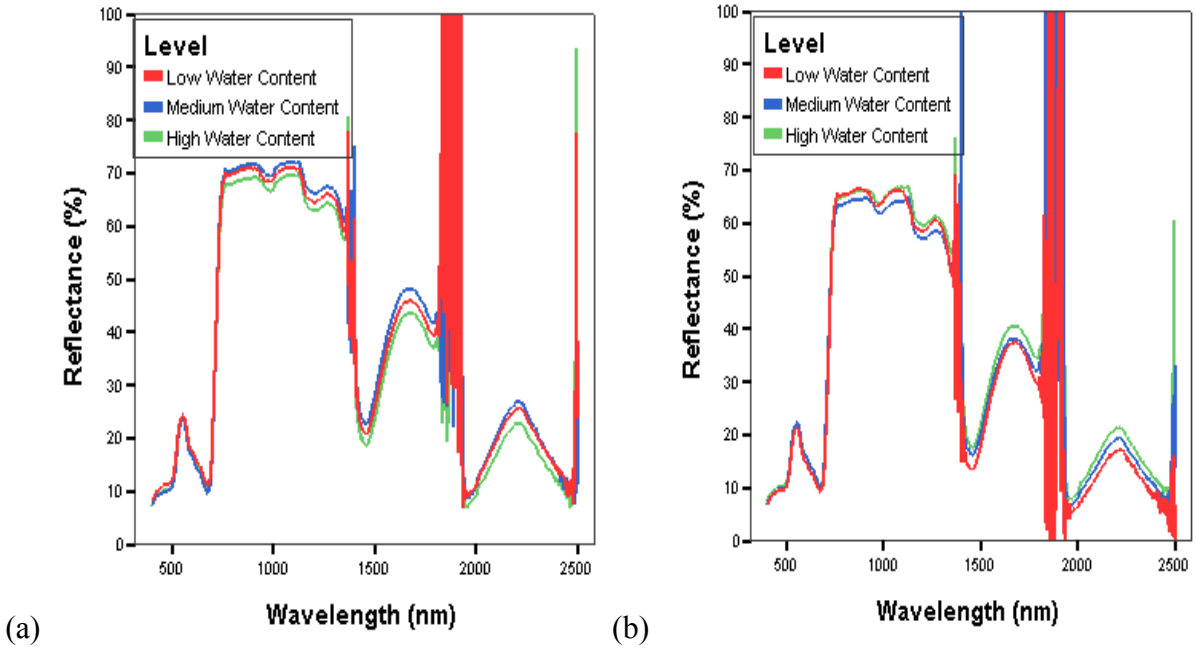
Fig. 4.11 Results of one-way ANOVA illustrating wavebands of (a) the spectrum range and (b) red-edge region using first derivative, that can significantly distinguish various damage levels on N37 at age 12 months. Dotted and solid horizontal lines indicate 0.01 and 0.05 significance levels (99 % and 95 % confidence limits), respectively.

The significant differences in leaf reflectance in the visible region (410 – 430 nm) are due to differences in leaf pigments such as chlorophyll and N concentrations while differences in leaf reflectance in the SWIR (2010 – 2340 nm) are due to water absorption effects as well as some nutrients such as N. These bands are almost same as those discovered by Datt *et al.* (2006) who showed that an increase in reflectance with increasing severity of leaf spot disease and bacteria soft rot (1100 – 2300 nm) was caused by reduction of tissue moisture and drying of dead leaves.

A better understanding about specific regions of the electromagnetic spectrum that provide maximum content when utilized for distinguishing various *E. saccharina* damage levels has been gathered or gained. This was further proven by the significance of these bands in discriminating between various damage levels. Therefore, this understanding makes it realistic to investigate the same bands on field level and canopy level, which will later lead to utility of these bands on airborne and spaceborne levels. Figure 4.11b highlights that first order derivatives in wavebands around 715 nm were able to differentiate between various *E. saccharina* damage levels ($p < 0.05$).

4.2.2.5 Differences in Mean Leaf Reflectance as induced by Different Water Content Levels

Figure 4.12 illustrates the water stress levels of different sugarcane varieties. However, it was difficult to quantify water stress levels as watering regimes were uniform throughout the whole trial.



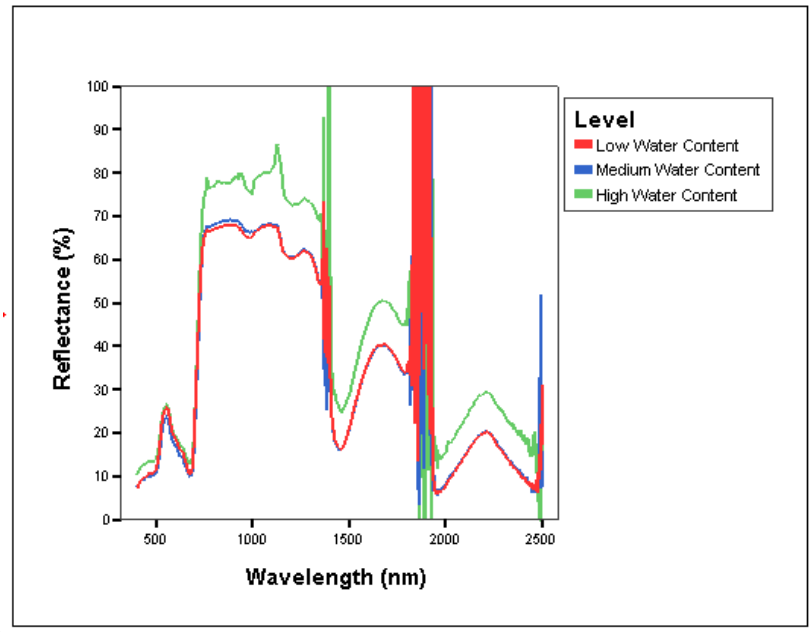


Fig. 4.12 Differences in mean leaf reflectance as induced by different water levels (Low water content = < 69%, Medium water content = 70 – 74% and High water content = > 75%) at different varieties. (a) N14, (b) N25 and (c) N37.

Figures 4.12a and b show that for N14 and N25, leaf reflectance could not clearly distinguish between different water levels. Among the three varieties, the N37 leaf reflectance distinguished between low water content and high water content successfully. Although, high water content reflected higher than low water content in most portion of the spectrum, the low water content had higher reflectance in the known water absorption bands centred on 1900 nm and 2500 nm (Figure 4.12c).

4.2.2.6 Correlations Between Leaf Reflectance and *E. saccharina* Stalk Damage as well as Number of *E. saccharina*

A Pearson's correlation was also performed to determine the relationship between *E. saccharina* stalk damage and reflectance as well as the number of *E. saccharina* and reflectance at each waveband at 12 months cane age at all varieties combined (Figure 4.13). Waveband 760 nm (r = 0.37) showed the strongest linear relationship between *E. saccharina* stalk damage and leaf reflectance compared to any bands throughout the spectrum (Figure 4.13a). Figure 4.13b indicates that wavebands 448 and 683 nm highlighted highest correlation between the number of *E. saccharina* and reflectance, r = 0.42 and r = 0.40, respectively.

These low correlations might be due to varietal influence as these correlations were undertaken for all varieties combined. As there have been low correlations between leaf reflectance and all biochemical concentrations as well as *E. saccharina* damage throughout all cane ages, an initiative was taken to perform these correlations at one cane variety (N37, as it was the most damaged by *E. saccharina* pest) at age 12 months (Figure 4.14).

Figure 4.14a validates that variety had a big influence on low correlations between reflectance and all biochemical concentrations as well as *E. saccharina* damage and number of *E. saccharina* larvae and pupae recovered from the stalks. This is shown by the fact that all variables had higher correlation coefficients ($r \geq 0.55$ and $r \leq -0.55$) with leaf reflectance at significant wavebands. Si concentration was an exception as it had r = 0.46, at 731 nm. Around the same region, N concentration and N/Si ratio had higher negative correlations, r = -0.52 at 704 nm and r = -0.56 at 713 nm, respectively. This further confirms that N absorption bands can be used to estimate N/Si ratio as well.

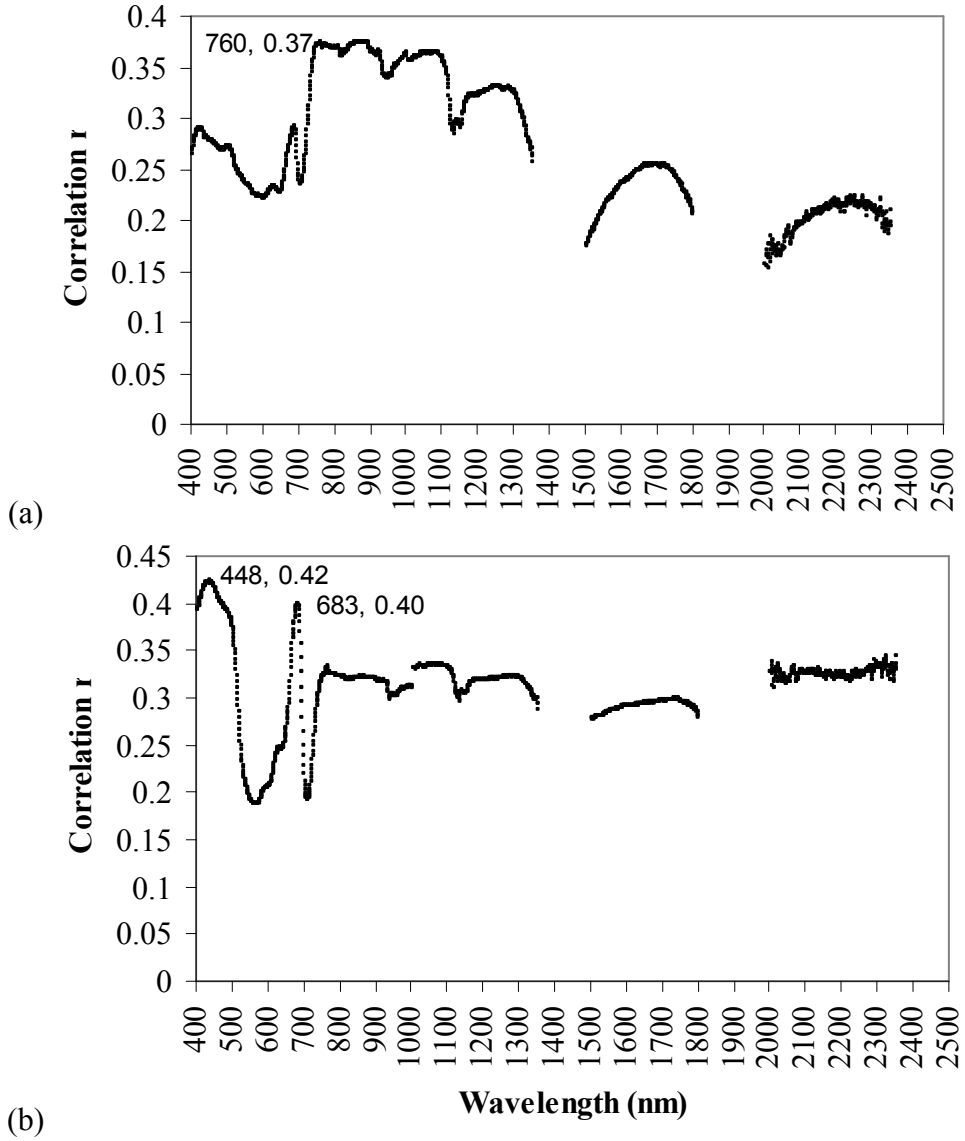
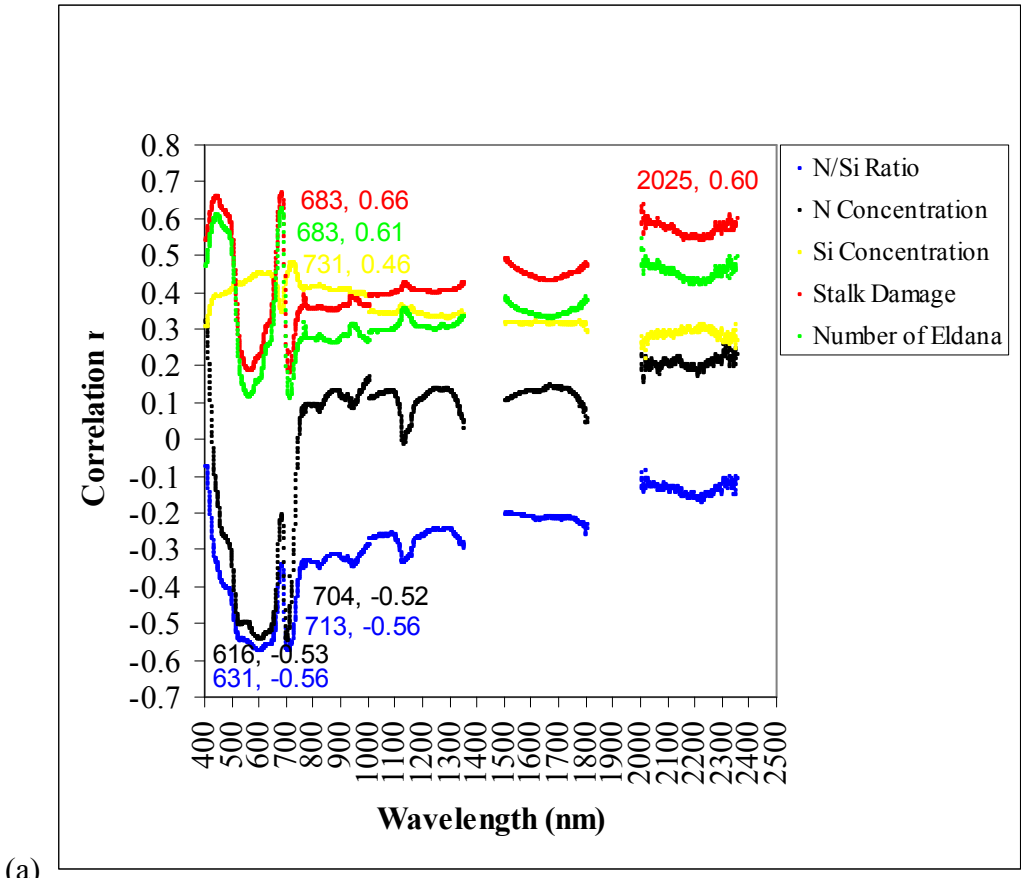


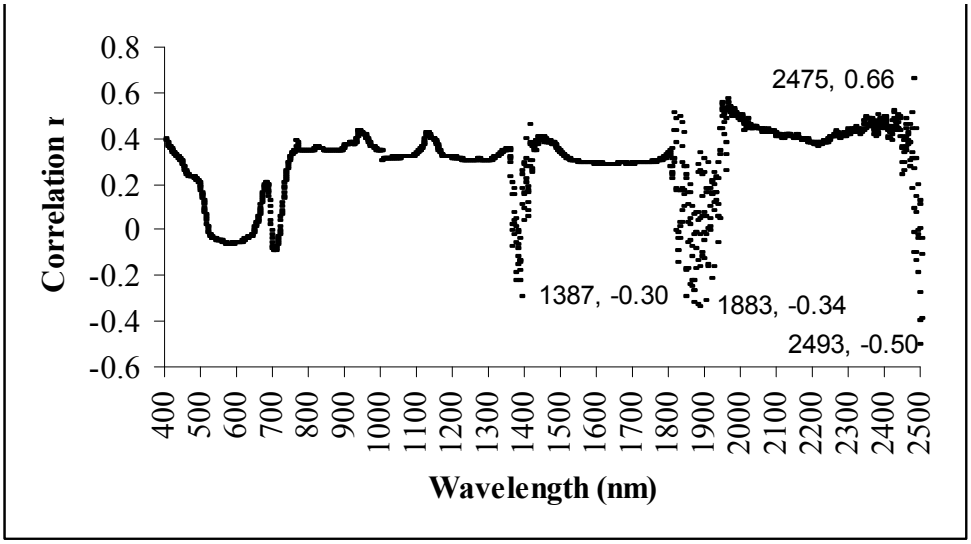
Fig. 4.13 Correlation between mean leaf reflectance and (a) Stalk damage by E. saccharina and (b) Number of E. saccharina larvae and pupae, indicated are wavelengths of peaks with high correlations on 12 months cane age at all varieties combined.

Eldana saccharina damage and number of *E. saccharina* larvae and pupae recovered from the stalks also had similar pattern, with highest positive correlations. This is expected as *E. saccharina* damage and number of *E. saccharina* larvae and pupae recovered from the stalks are highly correlated because *E. saccharina* larvae are the ones which bored the stalks ($r = 0.78$, $p < 0.01$, Table 4.2). These highest positive correlations in the visible region such around 430 and 683 nm and as well as in the SWIR (2000 – 2350 nm), that is at 2025 nm, indicates that increase *E. saccharina* stalk damage caused increase in leaf reflectance at these regions.

Water content was treated separately from other cane variables because noisy wavebands had to be included on the analysis while they were removed for other variables (Figure 4.14b). Reflectance from wavebands 2475 nm, 2493 nm, 1883 nm, and 1387 nm showed the highest correlation coefficients with leaf absolute water content ($r = 0.66$, $r = -0.50$, $r = -0.50$, $r = -0.34$ and $r = -0.30$, respectively) (Figure 4.14b). These results were encouraging as these wavebands were centered on the known water absorption bands, 1400 nm, 1900 nm and 2500 nm (Guyot, 1990; Lillesand and Kiefer, 2000; Kumar *et al*., 2003; ASD; 2006; Cho, 2007).



(a)



(b)

Fig. 4.14 Correlation between mean leaf reflectance and (a) N, Si Concentration, N/Si ratio, Stalk damage by E. saccharina and Number of E. saccharina and (b) water content, indicated are wavelengths of peaks with high correlations on N37 at age 12 months (n = 18).

4.2.2.7 Correlations Between Leaf Reflectance and Foliar Biochemical Concentrations

Correlations between reflectance and foliar biochemical concentrations were performed to investigate which wavebands can be used to estimate different biochemical concentrations (N concentration, Si concentration and N/Si ratio) at different cane ages (Figure 4.15, 4.16 and

4.17, respectively). Generally, positive correlation coefficients (r) indicate positive relationship between reflectance and biochemical concentration, that is when reflectance increases, biochemical concentration also increases, while negative correlation coefficients (r) show negative relationship, thus when reflectance decreases, biochemical concentration increases.

This simply means that high positive correlations show that there has been reflection peak because of the concerned biochemical concentration while the higher negative correlations simply show that there has been absorption peak due to the concerned biochemical concentration and these peaks are noted in Figures 4.15, 4.16 and 4.17 below. Figure 4.15a demonstrates that there were some correlation peaks in the shorter wavelengths (562 and 715 nm), however wavebands 1505 and 2059 nm gave the highest negative correlations for 7 months cane. These are around the known N absorption features centred on 1510 and 2060 nm, respectively (Kumar *et al.,* 2003).

Figure 4.16 results show similar trend with Figure 4.1b whereby there were highest correlations for Si, at 562 nm; r = 0.27 and 715 nm; r = 0.28 on 7 months cane, then there was a reduction on correlation at 446 nm; 0.17 and this increased a bit for 12 months cane (445 nm; r = 0.23). Figure 4.16c shows that in SWIR, bands 1506 nm; r = 0.33 and 2016 nm; r = 0.35, indicated that there were high correlations for foliar Si concentration.

Generally, throughout all cane ages, N/Si ratio had highest negative correlations at similar wavebands with N concentration in the shorter wavelengths (VNIR) (Figures 4.15, 4.16 and 4.17). For instance, both N and N/Si ratio had highest negative correlations at exactly the same wavebands 562 and 715 nm at age 7 months. At age 10 months, N had highest negative correlation at 669 nm and N/Si ratio at 647 nm. And lastly, at age 12 months, N showed highest negative correlation at 594 and 709 nm while N/Si ratio at 597 and 713 nm (Figures 4.15 and 4.17). This implies that same N absorption bands can be used to estimate N/Si ratio.

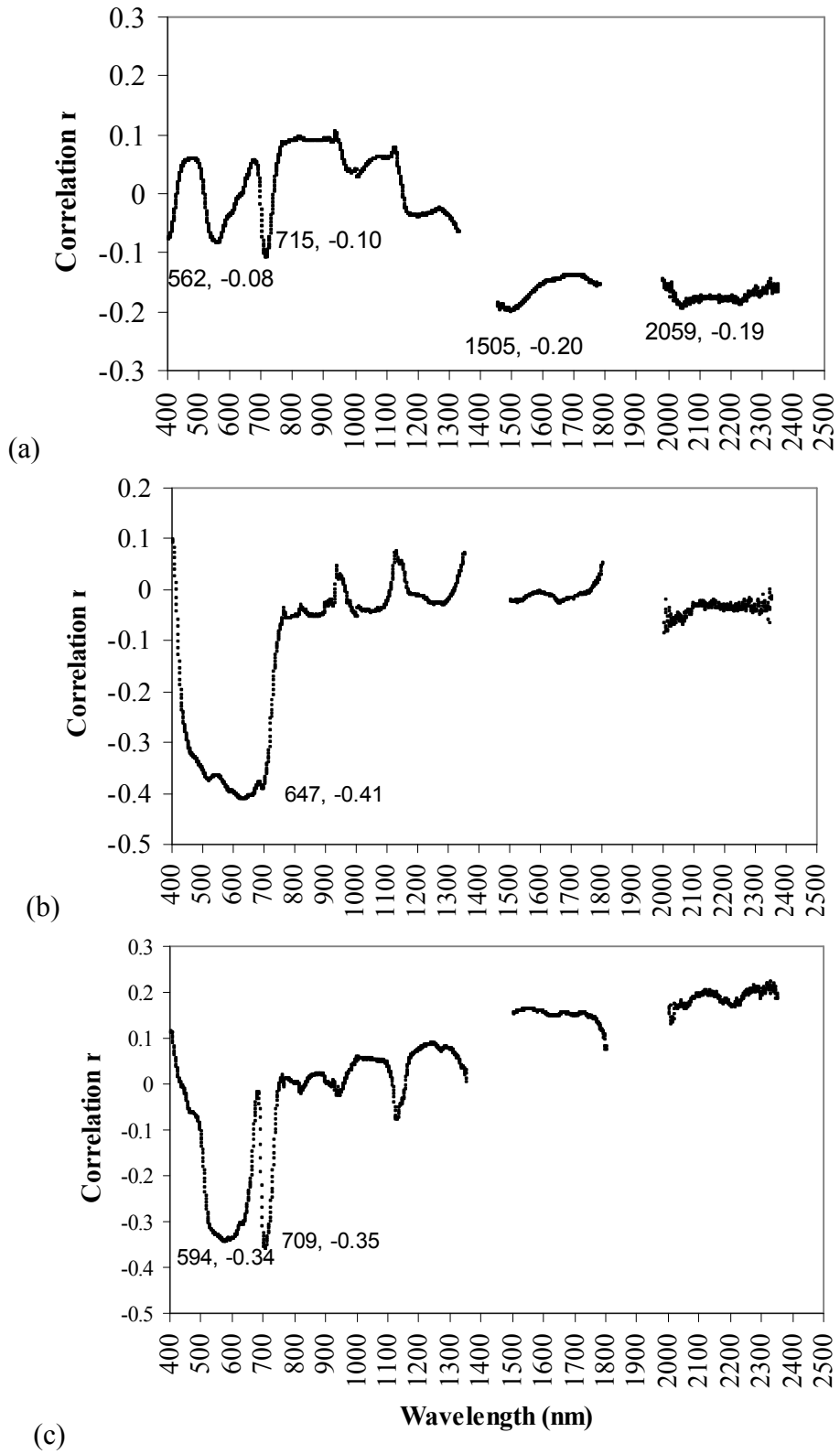


Fig. 4.15 Correlations between mean leaf reflectance and foliar N concentration, indicated are wavelengths of peaks with high correlations at different cane ages. (a) 7 months cane, (b) 10 months cane and (c) 12 months cane (n = 54).

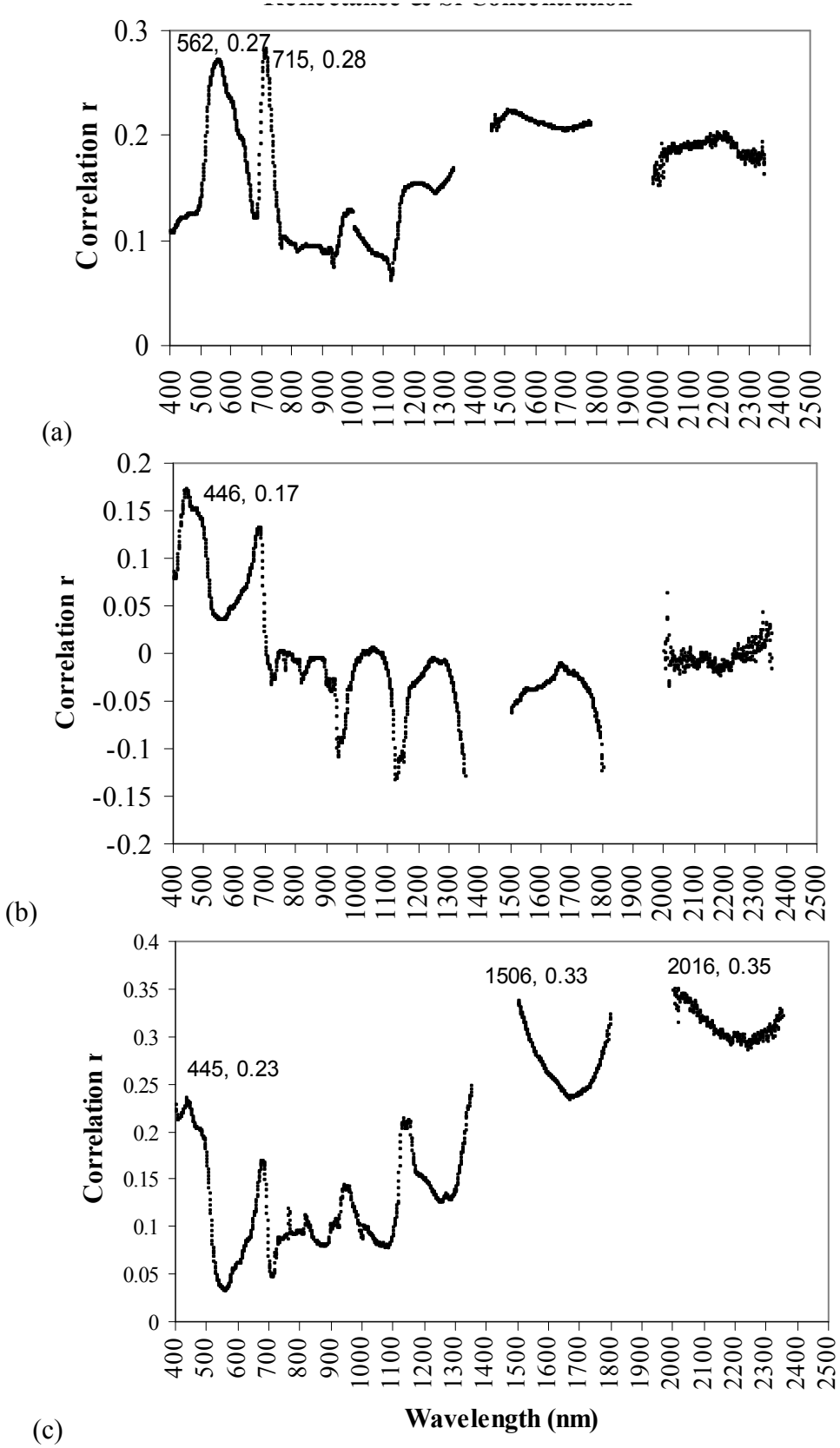


Fig. 4.16 Correlation between mean leaf reflectance and foliar Si concentration, indicated are wavelengths of peaks with high correlations at different cane ages (n = 54). (a) 7 months cane, (b) 10 months cane and (c) 12 months cane.

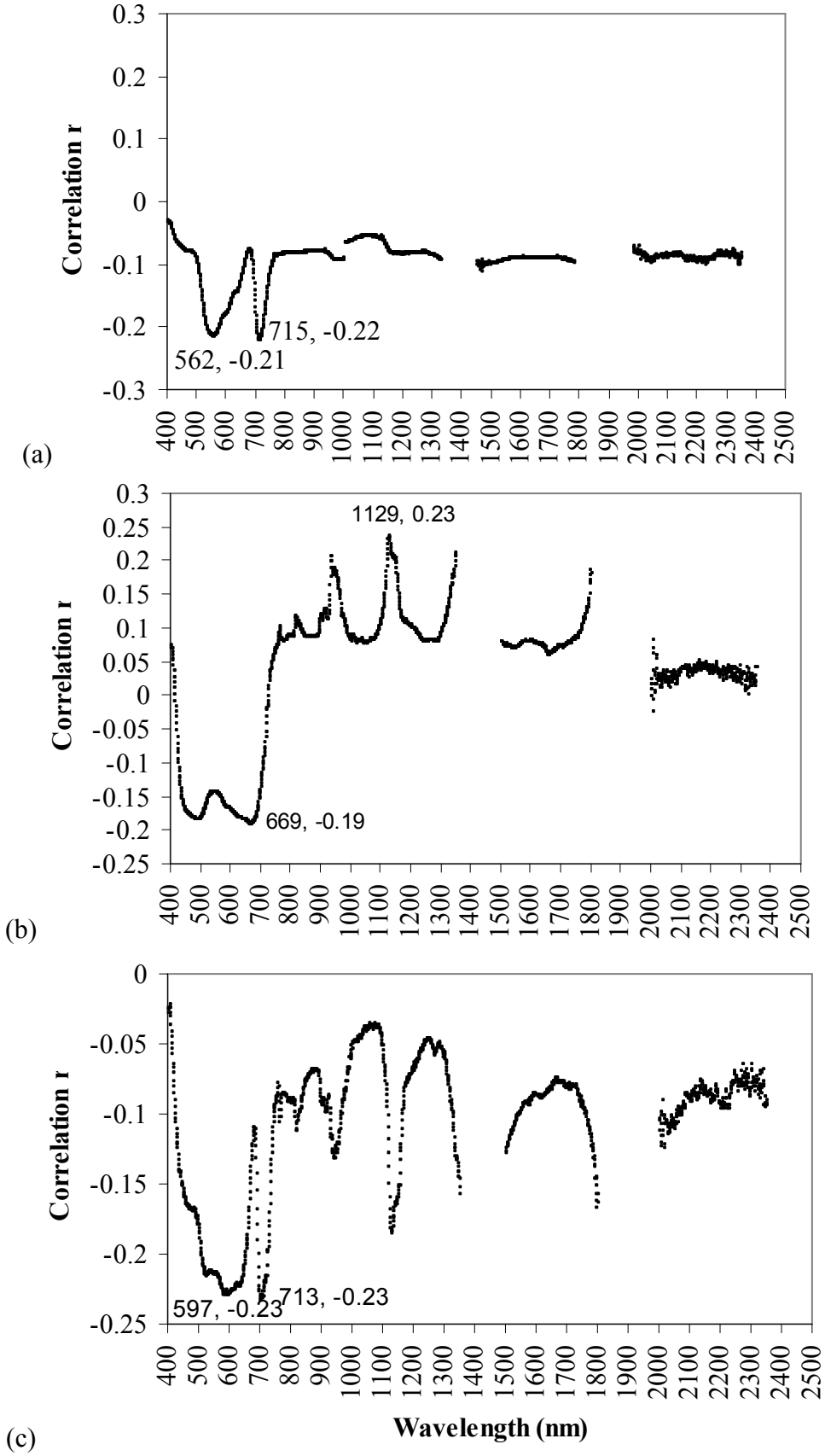


Fig. 4.17 Correlation between mean leaf reflectance and foliar N/Si Ratio, indicated are wavelengths of peaks with high correlations at different cane ages. (a) 7 months cane, (b) 10 months cane and (c) 12 months cane.

In contrast, Si concentration had highest positive correlations with reflectance in the same shorter wavelengths of VNIR region throughout all cane ages (Figure 4.16). This implies that Si concentrations had reflection peaks in the shorter wavelengths of VNIR region. This further supports that higher leaf reflectance in the shorter wavelengths are due to higher contents of silicates in the leaves (Alvarez-Añorve *et al.,* 2008). Although the relationship between leaf reflectance spectra and foliar biochemical concentrations was found to be weak throughout the electromagnetic spectrum, there are wavebands such as 562 and 715 nm which gave better correlations in all three foliar biochemical concentrations (N, Si and N/Si ratio) on 7 months cane (Figures 4.15, 4.16 and 4.17). That is negative correlations for N concentration and N/Si ratio and positive correlations for Si concentration.

Overall correlations between leaf reflectance and biochemical concentrations were found to be weak for all cane ages. There are two possible reasons for this, the first one might be the varietal influence as the correlations were performed on combined varieties. The second reason might be the fact that spectral measurement was taken on only one leaf while three leaves including the scanned one, were taken for chemical analysis.

4.2.2.8 Spectral Indices for Estimation of Foliar Biochemical Concentrations (N, Si and N/Si ratio) and *E. saccharina* Stalk Damage

Previously used narrow waveband spectral indices that are sensitive to leaf pigments such as N and chlorophyll as well as those sensitive to water status were tested in this study. A Pearson's correlation was performed to see relationship between N spectral indices and sugarcane dependent variables (N, Si concentration, N/Si ratio, and *E. saccharina* stalk damage) on variety N37 at age 12 months (Table 4.9). Table 4.9 demonstrates that all N spectral indices and first order derivatives had a highly significant correlation (with $P < 0.01$ in almost all of them) against leaf nitrogen concentration. However, NPCI and modified NDVI (R2200 and R2025) were exceptional as they were not statistically correlated to N concentration ($p > 0.05$). The modified NDVI of bands 2200 and 2025, instead showed highest significant negative correlation ($r = -0.62$, $p < 0.01$) when compared with *E. saccharina* stalk damage.

Table 4.9 Correlation of N spectral indices and sugarcane dependent variables (N, Si concentration, N/Si ratio, and *E. saccharina* stalk damage) on variety N37 at age 12 months.

N Indices	N Concentration	Si Concentration	N/Si Ratio	Eldana Stalk Damage
R810/R560	.716(**)	-0.25	0.45	-0.056
(R810-R560)/(R810+R560)	.718(**)	-0.266	.475(*)	-0.012
(R1075-R730)/(R1075+R730)	.726(**)	-0.355	.562(*)	0.134
D744	.738(**)	-0.115	0.34	0.205
D740	.807(**)	-0.146	0.388	0.195
D730	.774(**)	-0.096	0.333	0.236
mSR705	.696(**)	-0.236	0.428	0.113
(R750-R560/R750+R560)	.728(**)	-0.27	.478(*)	-0.009
R750/R700	.754(**)	-0.242	0.447	-0.111
PRI	.500(*)	-0.376	0.449	0.32
R740/R720	.762(**)	-0.299	.503(*)	0.005
(R1770-695/1770+695)	.685(**)	-0.171	0.382	0.084
(R2200-R2025)/(R2200+R2025)	-0.236	-0.098	-0.055	-.618(**)
D744/D2142	.658(**)	-0.281	0.394	-0.01
PSRI	.622(**)	-0.316	.497(*)	0.252
NPCI	-0.396	-0.119	-0.094	-0.058

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

It is important to note this index was developed by Abdel-Rahman *et al.* (2008a) for estimation of sugarcane N concentration on 6 -7 months N19 cane and was linearly related to N concentration ($R^2 = 0.87$). This further implies that *E. sachharina* damage on the stalk had some effects on leaf N concentrations. It is of great importance to highlight that both bands used in this index were found to be related to *E. saccharina* stalk damage in this study. Therefore, this implies *E. saccharina* damage on the cane stalk have an influence on N concentration. Table 4.9 also indicates that indices developed from the red-edge region as well as first derivatives showed poor correlations with *E. saccharina* stalk damage.

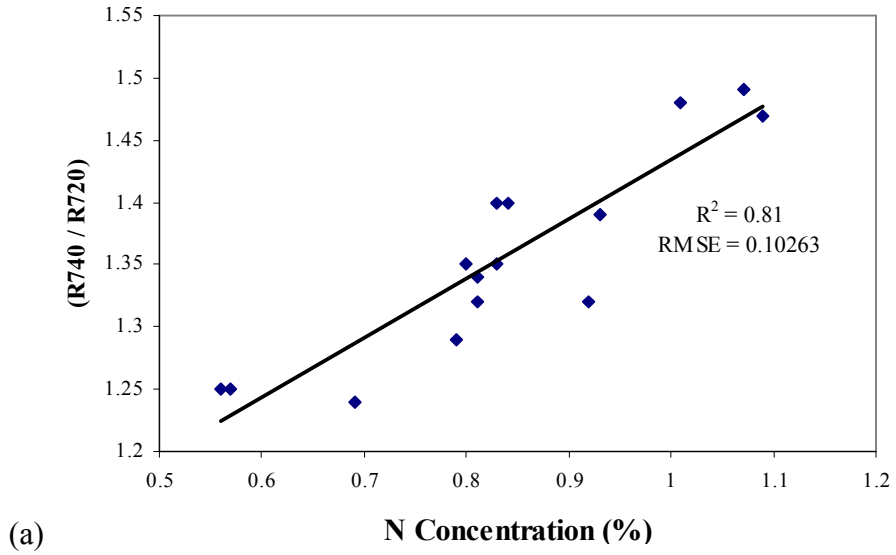
For foliar Si concentration, none of these N indices were significance on this variety as p was > 0.05 for all of them. This indicates that Si absorption bands are not related to N and chlorophyll absorption bands at this variety. Most N indices were significantly correlated to N/Si concentration. This further confirms that absorption bands for N concentration alone are same as N/Si ratio in the VNIR region (Figures 4.15, 4.16and 4.17, 4.14a). The results from the Table 4.9 made it realistic to try to test the regression models of spectral indices against biochemical concentrations and *E. saccharina* damage. Spectral N indices which showed highest significant correlations with N concentrations, N/Si ratio and *E. saccharina* stalk damage were further used to perform the following regression models (Figure 4.18).

Figure 4.18a shows that Red-edge Index (R_{740}/R_{720}) was linearly related to N concentration (R^2 = 0.81; RMSE = 0.10263). Modified NDVI $(R_{1075}-R_{730})/(R_{1075}+R_{730})$ was linearly related to N/Si ratio (R^2 = 0.67; RMSE = 1.508) (Figure 4.18b). Figure 4.18c indicates that *E. saccharina* stalk damage is linearly and negatively related to modified NDVI $(R_{2200}-R_{2025})/(R_{2200}+R_{2025})$ (R^2 = 0.69; RMSE = 19.351). This further proves that differences in leaf reflectance in the SWIR (2010 – 2340 nm) are due to N concentrations (Figure 4.16a). This implies that *E. sachharina damage* on the stalk had effects on leaf N concentrations.

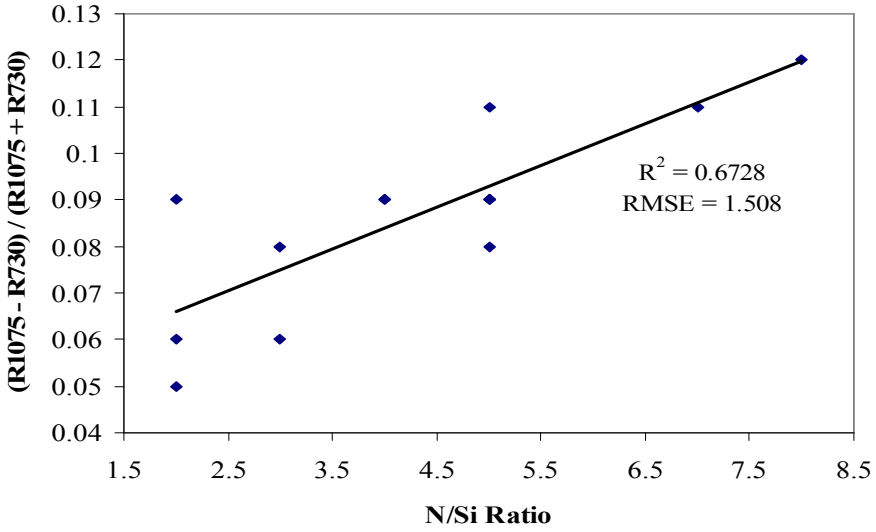
However, there were higher errors (RMSE) in these regression models (Figure 4.18), especially for *E. saccharina* stalk damage estimation (R^2 = 0.69; RMSE = 19.351), these might be due to the fact the bands used in the index are from SWIR (2000 – 2350 nm) which is characterized by water absorptions, lignin, N, starch and cellulose (Kumar *et al*., 2003). Therefore it is assumed that water stress could have some carry-over effects on these N absorption bands. The other possible reason for the high error is that spectral measurement was done only on one leaf from main per pot while stalk damage was done using all five plants in a pot.

The key finding is that, *E. saccharina* stalk damage may be more accurately predicted by vegetation indices from narrow wavelengths located in the SWIR (2010 – 2300 nm) than bands located in visible, red-edge and NIR regions of the spectrum. Therefore, this result allowed the extension of controlled experiments to field level as well as airborne/ spaceborne for estimation of *E. saccharina* stalk damage through entire area of sugarcane fields. This means that prediction of *E. saccharina* stalk damage can be done reliably using hand held field spectroradiometry, hence regression model(s) built from this can be applied on reflectance spectra acquired at the same resolution from airborne or spaceborne hyperspectral sensors.

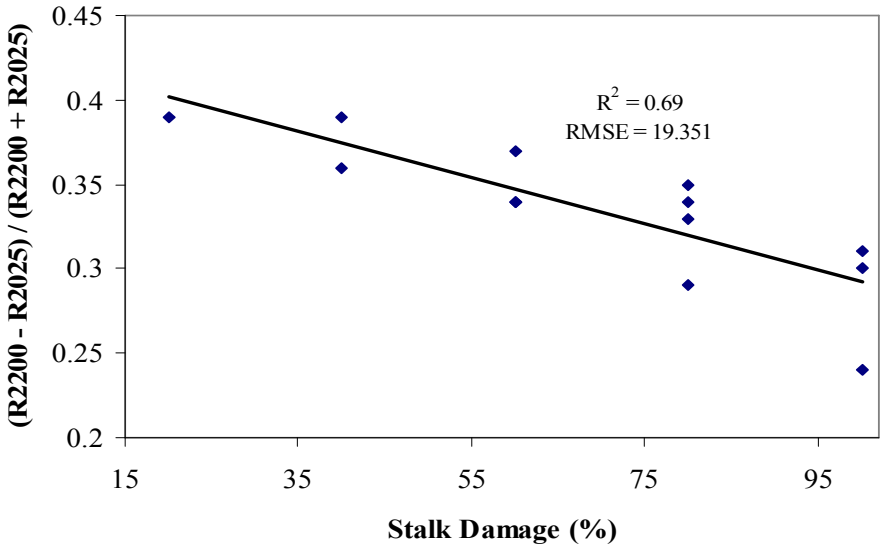
Among the tested VIs, including water indices, none of them was significantly correlated to water content for all three varieties. For Si concentration, significant correlation was only encountered on N25, hence an index showing highest significant correlation was used to develop a regression or relation model between Si concentration and leaf reflectance, that is $(R_{750}-R_{560})/(R_{750}+R_{560})$ (R^2 = 0.53, RMSE = 0.11817) (Figure 4.19).



(a)



(b)



(c)

Fig. 4.18 Linear regression models of spectral indices against biochemical concentrations and E. saccharina stalk damage on N37 at age 12 months (n = 18). (a) N Concentration, (b) N/Si ratio and (c) E. saccharina stalk damage.

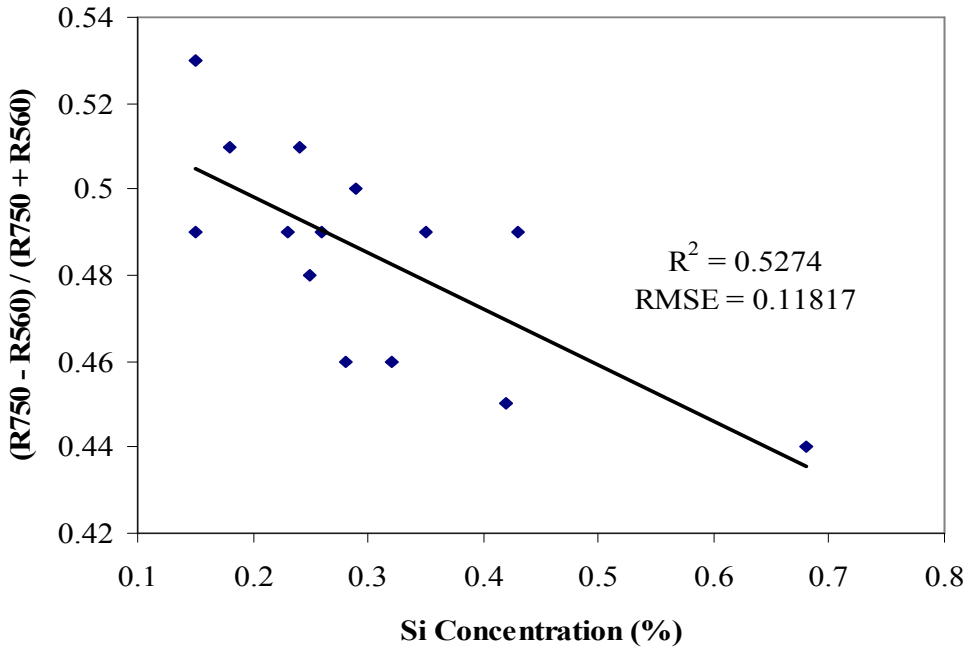


Fig 4.19 Linear regression model of spectral index against Si concentration on N25 at age 12 months (n = 18).

The higher error (RMSE) indicates that N could have some influence on this regression as it used N index. However, further studies are required to validate this and quantify Si concentration using spectral reflectance data from sugarcane leaves in general.

4.2.2.9 Varietal Discrimination by Leaf Reflectance at Different Ages as well as at All Ages Combined

One way ANOVA was performed to confirm that different varieties (N14, N25 and N37) had significant influence on leaf reflectance (VNIR region) at different cane ages (3, 7, 10 and 12 months) as well as at combined ages (Table 4.8). Figure 4.20 illustrates that different varieties had statistically significant effects on VNIR reflectance at ages 3, 7 and 12 months ($p = 0.002$, $p = 0.019$ and $p = 0.006$, respectively) (see Table 4.10). In all these ages, N14 reflected higher followed by N37 and lastly N25 in the NIR region (Figure 4.20a, b and d).

An exceptional case was found at age 10 months where there was no significance difference between the three cane varieties ($p = 0.676$) (see Table 4.10 and Figure 4.20c). This might be due to the that spectral measurements for 10 months cane were taken immediately after the cane plants had been subjected to water stress and also after N application termination on the cane trial. Figure 4.20 further confirms that the three varieties were statistically and significantly different ($p = 0.014$, Table 4.11) in VNIR reflectance at combined ages still with

N14 having highest NIR reflectance, then N37 and N25 with the lowest reflectance. Generally, these findings validate that reflectance from hyperspectral data can be used to discriminate sugarcane varieties (Apan *et al.,* 2004a; Galvão *et al.*, 2005).

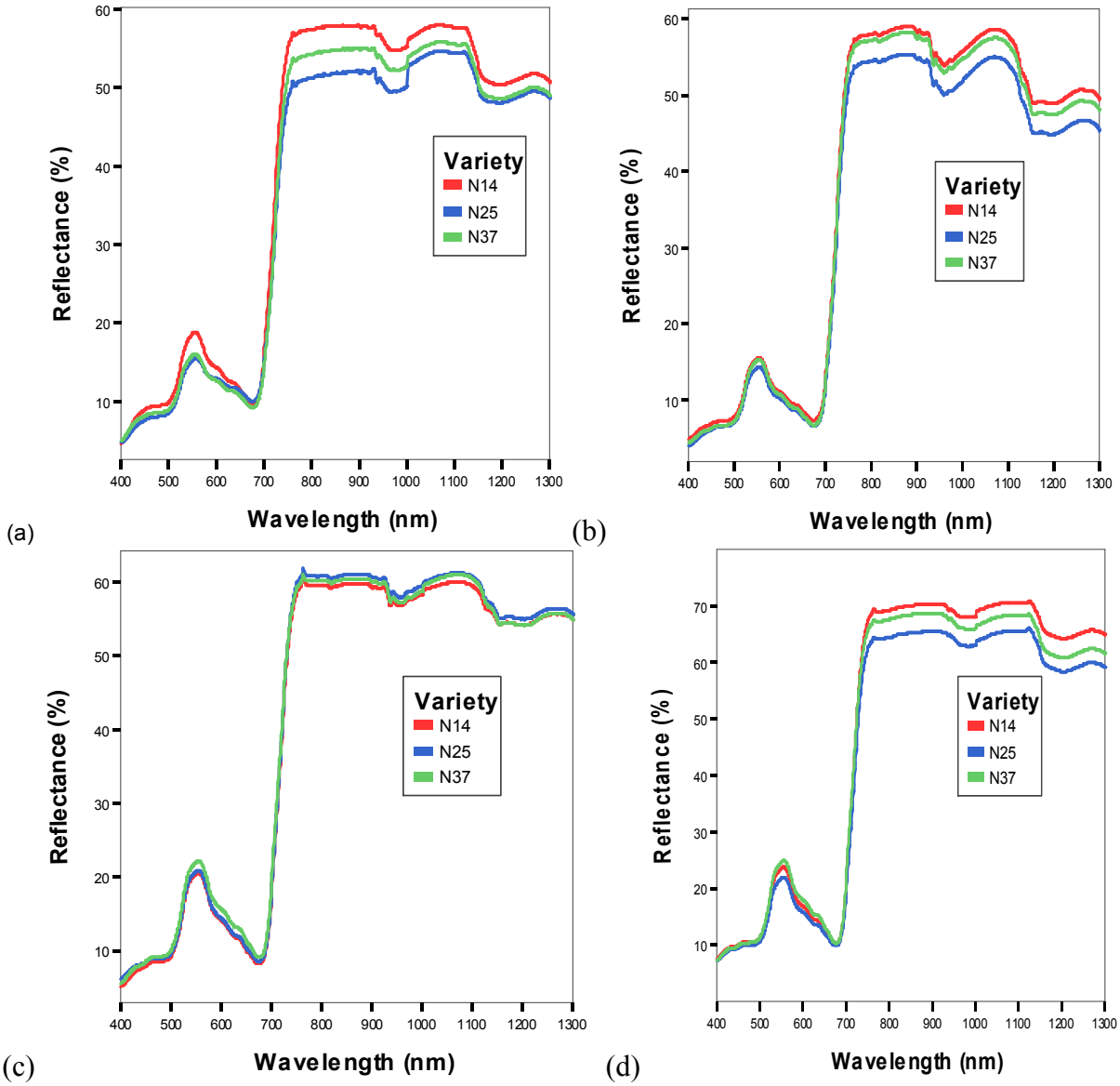


Fig.4.20 Relationship between mean leaf reflectance and different cane varieties (N14, N25 and N37) in visible-NIR portion of spectrum range (400 – 1300 nm) at different ages. (a) 3 months cane, (b) 7 months cane, (c) 10 months cane and (d) 12 months cane.

Table 4.10 Results of ANOVA showing if VNIR reflectance is affected by different cane varieties (N14, N25 and N37) at different cane ages.

Sugarcane Age	F	Sig.
3 months old cane	6.125	.002
7 months old cane	3.944	.019
10 months old cane	.392	.676
12 months old cane	5.137	.006

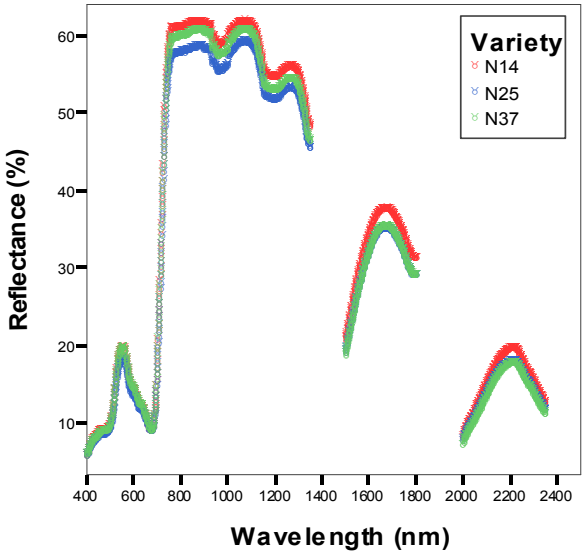


Fig. 4.21 Mean leaf spectral reflectance curve, whole spectrum without noisy wavebands, showing different varieties (N14, N25 and N37) at all ages combined.

Table 4.11 Results of ANOVA showing if whole spectrum as well as VNIR reflectance is influenced by different cane varieties (N14, N25 and N37) at all ages combined.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	4.279	.014

4.2.2.10 Relationship Between Mean Leaf Reflectance and Sugarcane Age

An initiative was taken to investigate whether the cane age can influence leaf spectral reflectance by running an ANOVA. Figures 4.22a, b and c illustrates that there were highly significant difference on leaf reflectance brought by different cane ages ($p < 0.0001$) (Table 4.12). There was significant increasing trend in leaf reflectance with increasing cane age, and also more pronounced in red-edge maximum inflection points from first derivative reflectance, thus 12 months cane had the highest reflectance throughout the spectrum range followed by 10 months cane (Figures 4.22a and c). However, there was an overlapping reflectance behavior between 3 and 7 months cane throughout the spectrum (Figures 4.22a and c).

Figure 4.22c shows that maximum red-edge peaks (REPs) for 3 months and 7 months cane were around 725 nm while for 10 and 12 months cane were near 720 nm. This indicates that REPs for 10 and 12 months cane as well as their red-edge slopes shifted towards shorter

wavelengths, resulting in the blue shift while those for 3 and 7 months moved towards longer wavelengths giving rise to the red shift (Figures 4.22b and c). These shifts were highly significant with $p < 0.0001$ (Table 4.12). This simply means that younger cane leaves have higher chlorophylls (and or N) and water content than older cane plants.

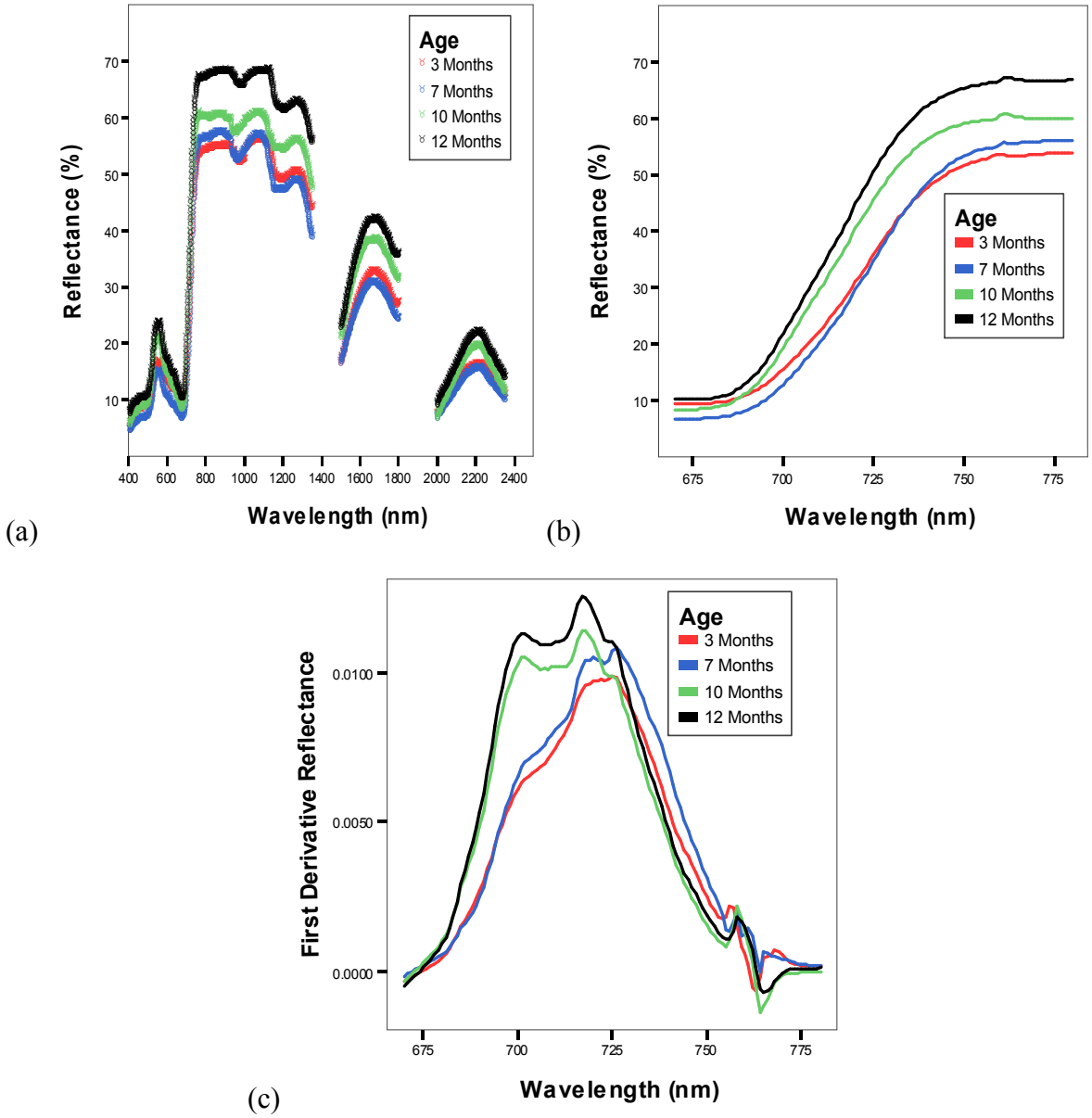


Fig. 4.22 Relationship between mean leaf reflectance and different sugarcane ages (3 months, 7 months, 10 months and 12 months). (a) whole spectrum without noisy wavebands, (b) and (c) Red-edge region.

Table 4.12 Results of ANOVA showing if leaf reflectance from the whole spectrum as well as from different portion of the spectrum is influenced by different cane ages.

Portion of Spectrum Range	F	Sig.
Whole spectrum range	70.760	.000
Red-edge (670 – 780 nm)	6.769	.000

4.3 General

As recent studies showed that hyperspectral remote sensing can offer an opportunity to monitor pests and diseases rapidly and non-destructively in agricultural crops, for example (Apan *et al.*, 2004; 2005; Mirik *et al.*, 2006a; b; Datt *et al.*, 2006; Abdel-Rahman *et al*., 2008b), the findings from this study further confirm that hyperspectral data with its narrow sensitive wavebands successfully discriminated between healthy and various *E. saccharina* damaged cane under controlled conditions.

Although hyperspectral data could distinguish between healthy and various *E. saccharina* stalk damage levels at combined varieties, it important to indicate that the best results were obtained when focusing on each variety as different varieties had different spectral reflectance (Apan *et al.,* 2004a; Galvão *et al.,* 2005), this was also confirmed in this study. In addition, different varieties had different *E. saccharina* susceptibility and different uptake of nutrients especially Si which increased resistance of varieties to *E. saccharina* stalk damage. This was illustrated by the fact that the most *E. saccharina* stalk damaged cane variety (N37) showed best significant results throughout the whole spectrum when distinguishing between healthy and various *E. saccharina* stalk damage levels. The successful estimation of *E. saccharina* stalk damage from hyperspectral data was also from this variety as indicated earlier.

The strongest positive relationship between *E. saccharina* stalk damage and leaf reflectance on N37 cane variety was found in the visible region, that is at 430 nm (blue); r = 0.65 and at 683 nm (red); r = 0.66 (Figure 4.14a). These positive correlations indicate that increase in *E. saccharina* infestations, increased leaf reflectance at these regions. This validates statement by Nilsson (1995) that any physiological stress, pest/disease, or reduced photosynthetic rate (P_n), results in increase in reflectance in the red and blue regions. The reflectance from green portion of the visible region was not related to *E. saccharina* stalk damage as r = 0.18 (Figure 4.14a). These findings are related to those of Apan *et al.* (2004b) and Datt *et al.* (2006), where there was higher significant reflectance on orange rust diseased cane than on healthy cane and higher reflectance on sunburnt lettuce than on healthy lettuce, respectively, at the red region (680 nm). However, in contrary to one of the findings by Datt *et al.* (2006) which indicated that reflectance from visible region showed that the largest differences between healthy and silverleaf affected pumpkin leaves, this study demonstrated that visible region had the least reflectance differences between healthy and various *E. saccharina* infested cane.

The fact that N is highly related to chlorophyll (Mutanga *et al.,* 2003; Zhao *et al.,* 2005), the significant movements of the red-edge slope and REP caused by various *E. saccharina* stalk damage levels are believed to affect N concentrations. For instance, severe *E. saccharina* stalk damage level caused the red-edge slope and the REP to significantly shift to the shorter wavelengths (blue shift), which indicated that there was reduction in chlorophyll concentrations hence N concentration. These results are encouraging as they are related to previous findings (Apan *et al.,* 2005; Datt *et al.,* 2006; Abdel-Rahman *et al.,* 2008b) dealing with effects of different pests and diseases under different plants at the red-edge.

In general, NIR showed the highest separability between healthy and various *E. saccharina* stalk damage levels at combined varieties as well as at each variety, especially N37 which was the most damaged variety. The results highlighted that in the NIR region severely *E. saccharina* damaged cane had the highest leaf reflectance followed by medium *E. saccharina* damaged cane. This implies that *E. saccharina* damage on the cane stalks did not break down cell structures in the cane leaves as this could have been noticed by a decrease in leaf reflectance in the NIR region which is associated with breakdown of leaf cell structures (Apan *et al.,* 2004b; 2005).

In the SWIR (2000 – 2350 nm) particularly band 2025 nm, had highest positive correlation which indicated that increase in *E. saccharina* stalk damage caused increase in leaf reflectance at this region. There was also highly significant differences ($p < 0.0001$, Table 4.5 and Figure 4.7) in leaf reflectance as influenced by various *E. saccharina* stalk damage levels in the SWIR (1500 – 1800 nm), with highest significant reflectance peaks for all *E. saccharina* damage levels centered on 1660 nm. Severe *E. saccharina* stalk damage had the highest reflectance peak. This was similar to findings by Apan *et al.* (2005) where the same band (1660 nm) from the range of 1590 – 1766 nm showed significant reflectance peak when discriminating healthy eggplant from damaged eggplant leaves by 28-spotted ladybird *(Epilachna vigintioctopunctata)*. Also, Apan *et al.* (2004b) indicated the key role played by SWIR wavebands (1660 – 2200 nm) in discrimination of healthy and orange rust diseased cane crops and further used band 1660 combined with other bands to indices which successfully discriminated healthy and orange rust diseased cane crops than any other indices. Results further show that wavebands 2010 – 2340 nm could significantly distinguish between the *E. saccharina* damage levels ($p \leq 0.05$) (Figure 4.11a). The significance of bands 2010 – 2340 nm further validates the higher correlations between leaf reflectance and *E. saccharina*

stalk damage which ranged from r = 0.5 – 0.6 in bands 2000 – 2350 nm (Figure 4.14). The significant differences in leaf reflectance in the SWIR (2010 – 2340 nm) are due to water absorption effects as well as some pigments such as N. These bands are almost same as those discovered by Datt *et al.* (2006) who showed that an increase in reflectance with increasing severity of leaf spot disease and bacteria soft rot (1100 – 2300 nm) was caused by reduction of tissue moisture and drying of dead leaves.

Overall, the spectral reflectance of severe *E. saccharina* stalk damage level was higher throughout the spectrum, thus from Visible-NIR-SWIR regions. This further validates that stress (biotic or abiotic stress) increases reflectance from 1300 to 2500 nm as well as in the range of 400 to 1300 nm (Carter, 1991; 1993). However, this is in contrary to some previous findings (Apan *et al.,* 2004b; 2005; Xu *et al.,* 2007; Yang *et al.,* 2008); where NIR reflected lower for infested or diseased plants than healthy plants due to breaking of cell saps in the leaves.

Reflectance based remote sensing techniques for pest identification capitalizes on the fact that most pests affect the outwards appearance of a plant in a particular manner either within the visible or outside the visible spectrum (Abdullah and Umer, undated). In addition, pathogens and pests can induce physiological stresses and physical changes in plants which can directly or indirectly affect reflectance properties of plants (Nilsson, 1995; Apan *et al.,* 2005; Datt *et al.,* 2006; Mirik *et al.,* 2006a). These were confirmed in this study as effects of *E. saccharina* damage on sugarcane leaves were not known but were picked up by leaf reflectance.

One of the N indices tested in this study, modified NDVI $(R_{2200}-R_{2025})/(R_{2200}+R_{2025})$, showed highest significant correlation with *E. saccharina* stalk damage on N37 (Table 4.9). This modified NDVI successfully estimated *E. saccharina* stalk damage on N37 from hyperspectral data with determination coefficient (R^2 = 0.69; RMSE = 19.351) (see Figure 4.18c). The higher RMSE was assumed to be the effects of water absorptions, lignin, starch and cellulose, more especially water stress which could have some carry-over effects, as bands used on this index were from SWIR (2000 – 2350 nm) which is characterized by water absorptions, lignin, N, starch and cellulose (Kumar *et al*., 2003). As *E. saccharina* infestations is related to water stress, it is assumed that formulation and development of *E. saccharina*-Water Stress Indices can be the best for estimation and detection of *E. saccharina*, possibly with low errors (RMSE). The other possible reason for the high error is that spectral

measurement was done only on one leaf from main per pot while stalk damage was done using all five plants in a pot.

This index $(R_{2200}-R_{2025})/(R_{2200}+R_{2025})$, was developed by Abdel-Rahman *et al.* (2008a) for estimation of sugarcane N concentration on 6 -7 months N19 cane and was linearly related to N concentration ($R^2 = 0.87$). This further implies that *E. sachharina* damage on the stalk had some effects on leaf N concentrations. It is of great importance to highlight that both bands used in this index were found to be related to *E. saccharina* stalk damage in this study. For instance, band 2025 nm showed one of the highest correlations between *E. saccharina* stalk damage and leaf reflectance on N37 (r = 0.6) (Figure 4.14a). On the other side, one of the highest reflectance peaks for all *E. saccharina* damage levels in the SWIR region were centred on 2200 nm (Figure 4.7f) on combined varieties. More importantly, both bands (2025 and 2200 nm) were within the range of 2010 – 2340 nm which significantly distinguished between the *E. saccharina* damage levels ($p \leq 0.05$) on N37. However, indices developed from the red-edge region as well as first derivatives such as Red-edge index, D740 and D730, showed poor correlations with *E. saccharina* stalk damage and water content. These confirm findings by Apan *et al.* (2004b) and Imanish *et al.* (2004), respectively.

The key finding is that, *E. saccharina* stalk damage may be more accurately predicted by vegetation indices from narrow wavelengths located in the SWIR (2010 – 2300 nm) than bands located in visible, red-edge and NIR regions of the spectrum. Therefore, this result allowed the extension of controlled experiments to field level as well as airborne/ spaceborne for estimation of *E. saccharina* stalk damage through entire area of sugarcane fields. This means that prediction of *E. saccharina* stalk damage can be done reliably using hand held field spectroradiometry, hence regression model(s) built from this can be applied on reflectance spectra acquired at the same resolution from airborne or spaceborne hyperspectral sensors.

Correlations between leaf reflectance and biochemical concentrations were found to be weak for all cane ages. There are two possible reasons for this, the first one might be the varietal influence as the correlations were performed on combined varieties. The second reason might be the fact that spectral measurements were taken on only one leaf while three leaves including the scanned one, were taken for chemical analysis. The second reason is similar to what was encountered by Ferwerda (2005) where spectral measurement was taken on one leaf

while chemical analysis was done on leaves from the whole canopy. The first reason was successfully validated by performing a varietal discrimination using leaf reflectance (Figure 4.20).

Although results showed that Si concentration had more contribution than N concentration in N/Si ratio, N/Si ratio had highest negative correlations at similar wavebands with N concentration in the shorter wavelengths (VNIR), where Si concentration had positive correlations (Figures 4.15, 4.16 and 4.17). This implies that same N absorption bands can be used to estimate N/Si ratio. Similar findings were obtained by Ferwerda (2005) where foliar N concentration showed regions of highest correlations similar to those of N/P ratio. This was further confirmed by successful estimation of N/Si ratio using N indices. One of the tested N indices, Modified NDVI $(R_{1075}-R_{730})/(R_{1075}+R_{730})$ (Zhao *et al.,* 2005), showed the highest significant correlation with N/Si ratio and was linearly related to N/Si ratio ($R^2 = 0.67$; RMSE = 1.508) (Figure 4.18b). Since, N/Si ratio has been found to be important for monitoring of *E. saccharina* in sugarcane fields (Keeping and Meyer, 2005a), showing that sugarcane with N/Si ratio greater than are 2 associated with increasing risk of *E. saccharina* borer damage, this index can be used for early detection of *E. saccharina* damage or for identifying sugarcane that is prone to attack by *E. saccharina* in the South African sugarcane industry.

All the tested N indices showed high significant correlation coefficients ($r > 0.5$, $p < 0.01$ at most) when compared to actual leaf N concentrations. Even previously used first derivatives 730, 740 and 744 nm (Zhao *et al.,* 2005; Abdel-Rahman *et al.*, 2008a) had the highest correlations with N concentration. However, the Red-edge Index (R_{740}/R_{720}) which showed the highest correlation coefficient and was linearly related to N concentration ($R^2 = 0.81$; RMSE = 0.10263) (Figure 4.18a) could be used for estimation of N concentration in relation *E. saccharina* incidence without destructing the leaves from sugarcane plants.

Even though this study revealed that there were some wavebands from blue region which were sensitive to foliar N concentration, these bands are normally not considered for development of N spectral indices due to the overlapping of chlorophylls and carotenoids absorption peaks in this blue region of the spectrum (Ray *et al.,* 2006).

Correlations between Si concentration and leaf reflectance showed highest positive correlation coefficients in the shorter wavelengths of VNIR region throughout all cane ages

(Figure 4.16). This implies that Si concentrations had reflection peaks in the shorter wavelengths of VNIR region. This confirms that higher leaf reflectance in the shorter wavelengths are due to higher contents of silicates in the leaves (Alvarez-Añorve *et al.,* 2008). However, significant correlation between Si concentration and N indices was only encountered on N25, hence an index showing highest significant correlation was used to develop a regression or relation model between Si concentration and leaf reflectance, that is $(R_{750}-R_{560})/(R_{750}+R_{560})$ ($R^2 = 0.53$, RMSE = 0.11817) (Figure 4.19). The higher error (RMSE) indicates that N could have some influence on this regression as it used N index. However, further studies are required to validate this and quantify Si concentration using spectral reflectance data from sugarcane leaves in general as this was the first attempt.

The proposed method, (assessment, monitoring and detection of *E. saccharina* using remote sensing), is advantageous over the traditional (visual) method, recently done by Way and Goebel (2007), as it does not involve destructive sampling of cane stalks from the field and then longitudinally splitting them to allow assessing stalk damage as well as internodes damage by *E. saccharina* and counting the number of larvae and pupae found in the stalks which is labour intensive and time consuming. Therefore, this proposed method offers an opportunity to monitor *E. saccharina* pest rapidly and non-destructively throughout the whole growing season.

In addition, as the effects of *E. saccharina* damage on cane leaves are not yet and the ones that are known on the stalks show up after damage, this method could serve as an early detection of *E. saccharina* from foliar remote sensing before visual signs of damage on the stalks come out. Therefore this will lead to prompt decision making on time or before great losses of sucrose content are encountered.

This proposed method can overcome the issue of bias as only easily accessible areas will be surveyed on traditional method and sometimes after heavy rains water stays in the fields which prevents monitors and surveyors from getting into the fields hence this lead to late monitoring and detection of pests, of which it might be after great crop loss (Abdullah and Umer, undated; Apan *et al.*, 2005). However, the proposed method, remote sensing in *E. saccharina* detection and assessment, is not meant to take over traditional method, instead it can be used to supplement traditional or visual approaches for assessment, monitoring and

detection of disease and pest (*E. saccharina)* symptoms (Abdullah and Umer, undated; Apan *et al.*, 2005).

Limitations of this proposed method are based on the fact that hyperspectral remote sensing data needs to be captured during clear sunny days on the field. In addition, hyperspectral images and spectroradiometers are very expensive hence it is important to know which bands can estimate or detect certain pests and diseases, for example *E. saccharina*, to avoid processing the whole spectrum which increases costs and to buy spectroradiometers with only appropriate bands, e.g. VNIR spectroradiometer if leaf reflectance differences caused the effects of the pest is highly separable from this region.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The results from this study highlight that hyperspectral remote sensing using hand-held spectroradiometers can provide a means of rapid assessment and monitoring of *E. saccharina* of many sugarcane samples, non-destructively, within a short time. However, this needs further investigation in the field as it was done under controlled conditions.

Was leaf-level spectral reflectance of sugarcane able to detect infestation by E. saccharina?

In order to determine if leaf-level spectral reflectance of sugarcane can be used to detect infestation by *E. saccharina*, all the leaf spectral reflectance from cane that did not have any stalk damage by *E. saccharina* were averaged to yield healthy cane while all those from damaged cane stalks were averaged to give *E. saccharina* damaged cane. Results show that there were highly significant differences in leaf reflectance from healthy and *E. saccharina* damaged cane throughout the spectrum ($P < 0.001$). The *E. saccharina* stalk damaged cane reflected higher than healthy cane throughout the spectrum, with more separability in the NIR region.

The red-edge slope and REP of the region *E. saccharina* damaged cane shifted towards shorter wavelengths. This is known as blue shift. This indicates that *E. saccharina* stalk damage caused decrease in leaf chlorophyll and N concentrations as this shift is the result of low chlorophyll concentrations.

Did leaf-level spectral reflectance of different sugarcane varieties successfully distinguish between various levels of E. saccharina damage and water stress?

Although hyperspectral data could distinguish between healthy and various *E. saccharina* stalk damage levels for all varieties combined, it important to indicate that the best results were obtained when focusing on each variety as different varieties had different spectral reflectance (Apan *et al.,* 2004a; Galvão *et al.,* 2005), this was also confirmed in this study.

Therefore the most damaged variety N37 demonstrated clearly significant leaf reflectance differences between various *E. saccharina* severity levels.

In general, NIR showed the highest separability between healthy and various *E. saccharina* stalk damage levels at combined varieties as well as at each variety, especially N37 which was the most damaged variety. The results highlighted that in the NIR region severely *E. saccharina* damaged cane had the highest leaf reflectance followed by medium *E. saccharina* damaged cane. This implies that *E. saccharina* damage on the cane stalks did not break down cell structures in the cane leaves as this could have been noticed by a decrease in leaf reflectance in the NIR region which is associated with breakdown of leaf cell structures.

Overall, the results illustrated that severe *E. saccharina* infestation increased reflectance throughout the whole spectrum range (400 – 2500 nm) and caused a red-edge slope and REP to shift towards the shorter wavelength (blue shift) indicating reduction in leaf chlorophyll and N concentrations. In terms of water stress, it was difficult to detect water stress using remote sensing in this study as watering regimes were uniform throughout the trial. It would have been better if there were different water stress levels such as non-stressed (control) and various stress levels. However, N37, which was the most infested variety, showed that leaf reflectance could distinguish between different water stress levels.

Which hyperspectral narrow-wave bands did successfully detect sugarcane E. saccharina and estimate N/Si ratio?

One of the N indices tested in this study, modified NDVI $(R_{2200}-R_{2025})/(R_{2200}+R_{2025})$, showed highest significant correlation with *E. saccharina* stalk damage and successfully estimated *E. saccharina* stalk damage on N37 from hyperspectral data with determination coefficient (R^2 = 0.69; RMSE = 19.351). Although this index successfully detected *E. saccharina,* many indices, not necessarily N indices, using different wavebands combinations can still be developed to estimate and monitor this pest. More importantly, as *E. saccharina* infestations are related to water stress, it is suggested that *E. saccharina*-Water Stress Indices are developed which can estimate *E. saccharina*, possibly with low errors (RMSE).

On the other hand, one of the tested N indices, Modified NDVI $(R_{1075}-R_{730})/(R_{1075}+R_{730})$ (Zhao *et al.,* 2005), showed the highest significant correlation with N/Si ratio and was linearly

related to N/Si ratio (R^2 = 0.67; RMSE = 1.508) (Figure 4.21b). Since, sugarcane with N/Si ratio greater than are 2 associated with increasing risk of *E. saccharina* borer damage this index can be used for early detection of *E. saccharina* damage or for identifying sugarcane that is prone to attack by *E. saccharina* in the South African sugarcane industry. However, several field based and canopy level studies are needed for quantification and validation of this as well as other indices which can estimate N/Si ratio in relation to *E. saccharina and* water stress incidence.

Was estimation of leaf biochemical concentrations of N and Si in relation to E. saccharina and water stress incidence using hyperspectral data successful?

The Red-edge Index (R_{740}/R_{720}) had the highest correlation coefficient and was linearly related to N concentration (R^2 = 0.81; RMSE = 0.10263) hence this index could successfully estimate N concentration in relation to *E. saccharina* incidence and water stress incidence. However, no significant correlation between foliar Si concentration and N indices was found the most damaged variety N37, instead it significant correlation was found in the least damaged variety N25. The index $(R_{750}-R_{560})/(R_{750}+R_{560})$ was linearly related to Si concentration (R^2 = 0.53, RMSE = 0.11817) hence can be used to estimate Si concentration in relation to E. saccharina and water stress, but not on most damaged cane.

Generally, the results from this study encourage an investigation into the potential of both field and airborne/spaceborne level hyperspectral data for detecting and discriminating *E. saccharina* infestations throughout the entire South African sugarcane production as this study was performed under controlled conditions such as soil nutrients, watering, and artificial infestation of *E. saccharina*.

5.2 Recommendations

As results from this feasibility study highlight that hyperspectral remote sensing using hand-held spectroradiometers can provide a means of rapid assessment and monitoring of *E. saccharina* under controlled conditions, it is highly recommended that further research studies using the same ASD spectroradiometer are conducted at field and canopy levels in the whole South African sugarcane production. It is also recommended that these hand-held spectroradiometers are used in conjunction with GPS to map *E. saccharina* infestations in

sugarcane fields, without destructing the cane plants throughout the South African sugarcane production especially where *E. saccharina* infestations are a problem.

Results also showed that modified NDVI $(R_{2200}-R_{2025})/(R_{2200}+R_{2025})$ can successfully estimate and monitor *E. saccharina* in South African sugarcane. However, it is recommended that *E. saccharina*-Water Stress Indices are developed which can estimate *E. saccharina*, possibly with low errors (RMSE). Since sugarcane with N/Si ratio greater than 2 are associated with increasing risk of *E. saccharina* borer damage this index can be used for early detection of *E. saccharina* damage or for identifying sugarcane that is prone to attack by *E. saccharina* in the South African sugarcane production. It is highly recommended that, several wavebands and indices that can estimate N/Si ratio in relation to water stress incidence are developed in South African sugar production as this will provide a proper and rapid means of identifying sugarcane that is prone to attack by *E. saccharina* hence necessary steps such IPM measures can be undertaken on time.

It also recommended that Imaging spectroscopy (airborne or satellite borne) with same narrow sensitive wavebands as this ASD spectroradiometer such as Hyperion are tested in South African sugarcane production which for assessment and monitoring of *E. saccharina* pest unbiased, non-destructively, cost-effectively, without time-consuming and repeatedly throughout the whole season in the industry. This will then enable identifying and mapping of *E. saccharina* infestations in the South African sugarcane production which will, in conjunction with biophysical parameters such soil characteristics and weather conditions, aid in modelling and prediction of potential *E. saccharina* infestation sites.

It is recommended that airborne/spaceborne hyperpesctral imageries should be acquired in conjunction field based data captured using hand-held spectroradiometers as these are known to be reference or ground truth data for airborne/spaceborne imageries. That is, indices developed from non-imaging (spectroradiometry) data can be used to analyse images from these airborne/spaceborne sensors, hence monitoring of *E. saccharina* as well as other pests and diseases can be done in all fields throughout the entire South African sugarcane production.

Finally, it is recommended that more studies regarding hyperspectral data in sugarcane health, such as *E. saccharina* detection, are investigated so that when the forthcoming South African

spaceborne imaging spectrometer, Sumbandila Sat (ZASat-002, South African first satellite (Scholes and Annamalai, 2006)) comes specific wavebands for different sugarcane applications will be known. Development of spectral library for sugarcane plants with regard to cane health will be of great importance throughout the entire South African sugarcane production.

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APPENDICES

Appendix A

On a separate page.

Appendix A

Glasshouse trial
Nitrogen By Silicon By Variety

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	9	9	9	5	5	5	1	1	1	2	2	2	4	4	4	6	6	6	8	8	8	7	7	7	3	3	3
Rep1	V1	V3	V2	V1	V3	V2	V3	V1	V2	V2	V3	V1	V1	V3	V2	V1	V2	V3	V1	V2	V3	V3	V2	V1	V3	V1	V2
	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
	2	2	2	1	1	1	8	8	8	4	4	4	6	6	6	3	3	3	9	9	9	7	7	7	5	5	5
Rep2	V2	V1	V3	V1	V3	V2	V1	V2	V3	V3	V1	V2	V2	V1	V3	V2	V3	V1	V3	V2	V1	V1	V3	V2	V3	V2	V1

Design : Split Plot with N*S as whole plot treats and Variety as split plot treat

Whole plot treatments:

1	N1S0
2	N1S1
3	N1S2
4	N2S0
5	N2S1
6	N2S2
7	N3S0
8	N3S1
9	N3S2

Variety

V1	N 14
V2	N 25
V3	N 37

Nitrogen

N1	30ppm
N2	60ppm
N3	90ppm

Silicon

S0	0	calcium silicate	per pot	0ppm
S1	53g	calcium silicate	per pot	100ppm 225 t/ha
S2	106g	calcium silicate	per pot	200ppm 450 t/ha

Appendix B

Results of Factorial ANOVA showing significant differences in the foliar biochemical concentrations (N concentration, Si concentration and N/Si ratio) for 7 months old cane as influenced by independent variables (N treatment, Si treatment and variety) as well as their interactions.

Dependent Variable: N					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	0.185348	2	0.092674	3.762707	0.036186
Si Treatment	0.012193	2	0.006096	0.247519	0.782488
Variety	0.523748	2	0.261874	10.63248	0.000393
N Treatment * Si Treatment	0.071763	4	0.017941	0.728421	0.580373
N Treatment * Variety	0.064474	4	0.016119	0.654436	0.628833
Si Treatment * Variety	0.08563	4	0.021407	0.869173	0.495092
N Treatment * Si Treatment * Variety	0.052948	8	0.006619	0.268722	0.970788
Dependent Variable: Si					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	0.112	2	0.056	1.795	0.185
Si Treatment	1.018	2	0.509	16.26	0.000001
Variety	0.006	2	0.003	0.095	0.91
N Treatment * Si Treatment	0.379	4	0.095	3.027	0.035
N Treatment * Variety	0.031	4	0.008	0.247	0.909
Si Treatment * Variety	0.114	4	0.029	0.915	0.47
N Treatment * Si Treatment * Variety	0.361	8	0.045	1.442	0.225
Dependent Variable: N/Si Ratio					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	12.32281	2	6.161403	1.028097	0.371269
Si Treatment	64.85393	2	32.42696	5.410789	0.010566
Variety	18.7041	2	9.352049	1.56049	0.22839
N Treatment * Si Treatment	36.26988	4	9.067471	1.513005	0.226365
N Treatment * Variety	5.95297	4	1.488242	0.248329	0.908145
Si Treatment * Variety	32.85576	4	8.213941	1.370585	0.270302
N Treatment * Si Treatment * Variety	48.17437	8	6.021796	1.004802	0.455301

Appendix C

Results of Factorial ANOVA showing significant differences in the foliar biochemical concentrations (N concentration, Si concentration, N/Si ratio and water content) on 10 months old cane as influenced by factors (N treatment, Si treatment and variety) as well as their interactions.

Dependent Variable: N					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	0.734708	2	0.367354	7.176002	0.003167
Si Treatment	0.25878	2	0.12939	2.527547	0.098583
Variety	0.181338	2	0.090669	1.771154	0.189337
N Treatment * Si Treatment	0.166525	4	0.041631	0.813237	0.52783
N Treatment * Variety	0.131369	4	0.032842	0.641552	0.637496
Si Treatment * Variety	0.11188	4	0.02797	0.546372	0.703155
N Treatment * Si Treatment * Variety	0.200654	8	0.025082	0.489954	0.852519
Dependent Variable: Si					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	0.005393	2	0.002696	0.622222	0.544271
Si Treatment	0.104604	2	0.052302	12.06966	0.00018
Variety	0.042804	2	0.021402	4.938889	0.014862
N Treatment * Si Treatment	0.017919	4	0.00448	1.033761	0.407947
N Treatment * Variety	0.012519	4	0.00313	0.722222	0.584344
Si Treatment * Variety	0.008341	4	0.002085	0.481197	0.749273
N Treatment * Si Treatment * Variety	0.02677	8	0.003346	0.772222	0.630045
Dependent Variable: N/Si Ratio					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	29.99677	2	14.99838	2.432276	0.106845
Si Treatment	40.20004	2	20.10002	3.259604	0.053944
Variety	11.30346	2	5.651728	0.916536	0.411987
N Treatment * Si Treatment	23.90063	4	5.975157	0.968986	0.440602
N Treatment * Variety	14.66313	4	3.665783	0.594477	0.669637
Si Treatment * Variety	13.51957	4	3.379894	0.548115	0.701931
N Treatment * Si Treatment * Variety	26.065	8	3.258125	0.528368	0.824647
Dependent Variable: Water Content					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	1.814815	2	0.907407	0.024987	0.975345
Si Treatment	72.48148	2	36.24074	0.99796	0.381824
Variety	95.81481	2	47.90741	1.319225	0.284028
N Treatment * Si Treatment	79.2963	4	19.82407	0.545895	0.70349
N Treatment * Variety	145.6296	4	36.40741	1.00255	0.423417
Si Treatment * Variety	235.6296	4	58.90741	1.622132	0.197471
N Treatment * Si Treatment * Variety	36.25926	8	4.532407	0.124809	0.997644

Appendix D

Results of Factorial ANOVA showing significant differences in the foliar biochemical concentrations (N concentration, Si concentration, N/Si ratio, water content) and *E. saccharina* damage on 12 months cane age as influenced by independent variables (N treatment, Si treatment and variety) as well as their interactions.

Dependent Variable: N					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	0.52323	2	0.261615	5.622184	0.009094
Si Treatment	0.006925	2	0.003462	0.074409	0.928481
Variety	0.646844	2	0.323422	6.950433	0.003673
N Treatment * Si Treatment	0.051556	4	0.012889	0.276989	0.890255
N Treatment * Variety	0.048797	4	0.012199	0.262165	0.899611
Si Treatment * Variety	0.064955	4	0.016239	0.348977	0.8424
N Treatment * Si Treatment * Variety	0.263282	8	0.03291	0.707252	0.68273
Dependent Variable: Si					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	0.050948	2	0.025474	2.858687	0.0748
Si Treatment	0.183715	2	0.091857	10.30819	0.000472
Variety	0.127215	2	0.063607	7.137988	0.003247
N Treatment * Si Treatment	0.046663	4	0.011666	1.309123	0.29168
N Treatment * Variety	0.029163	4	0.007291	0.818163	0.524885
Si Treatment * Variety	0.004363	4	0.001091	0.122402	0.973223
N Treatment * Si Treatment * Variety	0.051393	8	0.006424	0.720906	0.67161
Dependent Variable: N/Si Ratio					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	50.86255	2	25.43128	11.59103	0.000232
Si Treatment	46.798	2	23.399	10.66476	0.000386
Variety	12.93092	2	6.465459	2.946816	0.069567
N Treatment * Si Treatment	12.70438	4	3.176096	1.447596	0.245615
N Treatment * Variety	9.11895	4	2.279737	1.039055	0.405371
Si Treatment * Variety	5.224169	4	1.306042	0.595266	0.669093
N Treatment * Si Treatment * Variety	11.40264	8	1.42533	0.649635	0.729601
Dependent Variable: Water Content					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	84.48148	2	42.24074	3.482443	0.045134
Si Treatment	28.48148	2	14.24074	1.174046	0.324401
Variety	330.037	2	165.0185	13.60458	8.19E-05
N Treatment * Si Treatment	228.0741	4	57.01852	4.700763	0.005213
N Treatment * Variety	21.18519	4	5.296296	0.436641	0.780954
Si Treatment * Variety	54.51852	4	13.62963	1.123664	0.366081
N Treatment * Si Treatment * Variety	129.5926	8	16.19907	1.335496	0.268813
Dependent Variable: *E. saccharina* damage					

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
N Treatment	181.1481	2	90.57407	0.164232	0.849386
Si Treatment	10785.59	2	5392.796	9.778416	0.000639
Variety	10577.37	2	5288.685	9.589638	0.000713
N Treatment * Si Treatment	1771.963	4	442.9907	0.803247	0.53384
N Treatment * Variety	4277.852	4	1069.463	1.939189	0.132688
Si Treatment * Variety	2186.741	4	546.6852	0.99127	0.429129
N Treatment * Si Treatment * Variety	2906.704	8	363.338	0.658818	0.722157