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The Agronomic Use and Application of Canopy Reflectance within the Visible and Near-Infrared Wavebands and its Relation with Nitrogen Fertilization in Energy Cane Production in Louisiana

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THE AGRONOMIC USE AND APPLICATION OF CANOPY REFLECTANCE WITHIN THE VISIBLE AND NEAR-INFRARED WAVEBANDS AND ITS RELATION WITH NITROGEN FERTILIZATION IN ENERGY CANE PRODUCTION IN LOUISIANA

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Abstract

Spectral vegetation index-based models that are used to estimate yield potential are commonly developed from the relationship between early-season crop canopy reflectance readings and actual yield obtained at harvest. Plant population stand can influence cane yield potential and nutrient requirement. This study was conducted at LSU AgCenter Sugar Research Station in St. Gabriel, LA to evaluate (1) the relation between estimated early season biomass yield and the spectral vegetation indices acquired at the same time, (2) nitrogen (N) response pattern between early-season biomass production and yield at harvest, and (3) the relationship between coefficient of variation (CV) among normalized difference vegetation index (NDVI) readings and stand population of cane planted as whole stalk and billets. Treatments were applied in split plots with a randomized complete block design with four replications. Varieties (Ho 02-113, US 72-114) and N application rates (0, 56, 112, and 224 kg N ha^{-1}) were assigned as main plots and sub-plots, respectively. Another experiment was conducted with planting schemes (whole stalks and billets) and varieties (Ho 02-113, US 72-114, Ho 06-9001, Ho 06-9002, L 01-299, and L 03-371) arranged as main and sub-plots, respectively. Biomass clippings and canopy spectral reflectance readings using Jaz® spectrometer were collected at three, four, and five weeks after N application (WAN). Results showed that early-season biomass yield and its canopy reflectance collected at the same time were correlated. Overall, the relationships between vegetation indices (VIs) and biomass were best described with quadratic model at four WAN. Reflectance from red wavelengths (670 and 690 nm) and VI computed from them consistently performed better than the reflectance from red-edge wavelengths in relating early-season biomass production. Variables collected at four and five WAN showed similar response pattern to variable N rates as with harvest. Under favorable weather, billet-planted cane produced higher initial plant population compared to whole stalk-planted cane in 2013. Negative

correlation was found between CV among NDVI and plant population. Coefficient of variation among red-based vegetation indices produced better correlation with plant population than those from different wavelengths. Variety had no effect on canopy spectral reflectance.

Chapter 1. Introduction

1.1 Background

Solar radiation is the main source of energy on the earth, and by the process of photosynthesis, plants convert this solar energy into chemical energy. Fossil fuels, such as coal, natural gas, and oil, are considered as the secondary source of solar energy and were synthesized through the anaerobic decomposition of organic matter over millions of years. The abundance of the fossil fuel has led to its high consumption (REN, 2012), while other energy sources have not been thoroughly explored. It is assumed that the increasing world population will increase global energy consumption by nearly 53% from 2008 to 2035 (EIA, 2011). According to United States Energy Information Administration (EIA, 2011), Saudi Arabia was the largest producer of the natural oil in 2012 followed by USA and Russia. Due to socio-political problems in Middle Eastern countries (Saudi Arabia, United Arab Emirates, Kuwait, Iraq, and Qatar) and in Africa (Nigeria, Angola, and Algeria), the oil price increased from $82 to $112 per barrel (~ 160 liter) within six months in 2011 (EIA, 2011). Under these circumstances, interest increased in alternative sources of energy opening up research opportunities. The production of different biofuel crops, such as energy cane (*Saccharum spp.*) could mitigate this problem to some extent. Unlike fossil fuels, biofuel can be produced from renewable resources (plant and animal sources) and can provide sustainability to the environmental system.

1.2 Different biofuel crops and their importance

Biodiesel and bioethanol are two types of biofuel products. Biodiesel is normally derived from animal fat and vegetable oil. It is produced by the esterification (reaction between organic acids and alcohols where the H^+ of the COOH group of acid is replaced by the functional group of alcohol) and trans-esterification (reaction between ester and alcohol where the functional groups become exchanged). Vegetable oil and animal fats are reacted with some alcohols

(commonly methanol) in the presence of strong alkali catalyst (normally sodium or potassium hydroxides) to produce glycerin and methyl ester (Gerpen, 2005) (Fig.1.1). The excess methanol can be recovered and recycled for biodiesel production. Glycerin can be used in pharmaceutical industries and methyl ester is the biodiesel.

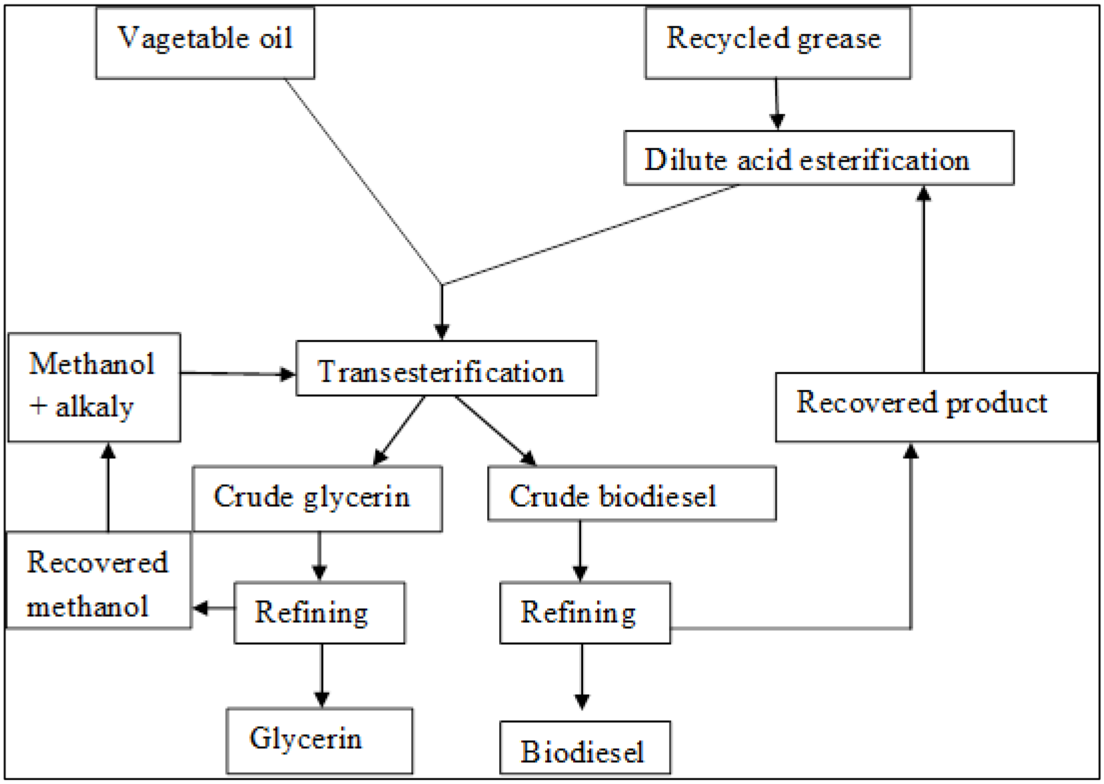

Figure 1.1. Flow chart showing basic technology of production of biodiesel (Cogeneration Technology, 2002).

Bioethanol is an alcohol, mostly derived from sugar and starch (first generation biofuel), lignocellulose biomass (second generation biofuel or cellulosic biofuel), and algae (third generation). Bioethanol production includes saccharification (conversion of complex sugars to simple sugars), fermentation (formation of sugars into alcohols by yeast), distillation (separation of ethanol from other liquid mixture), and dehydration (removal of water) (Fig. 1.2).

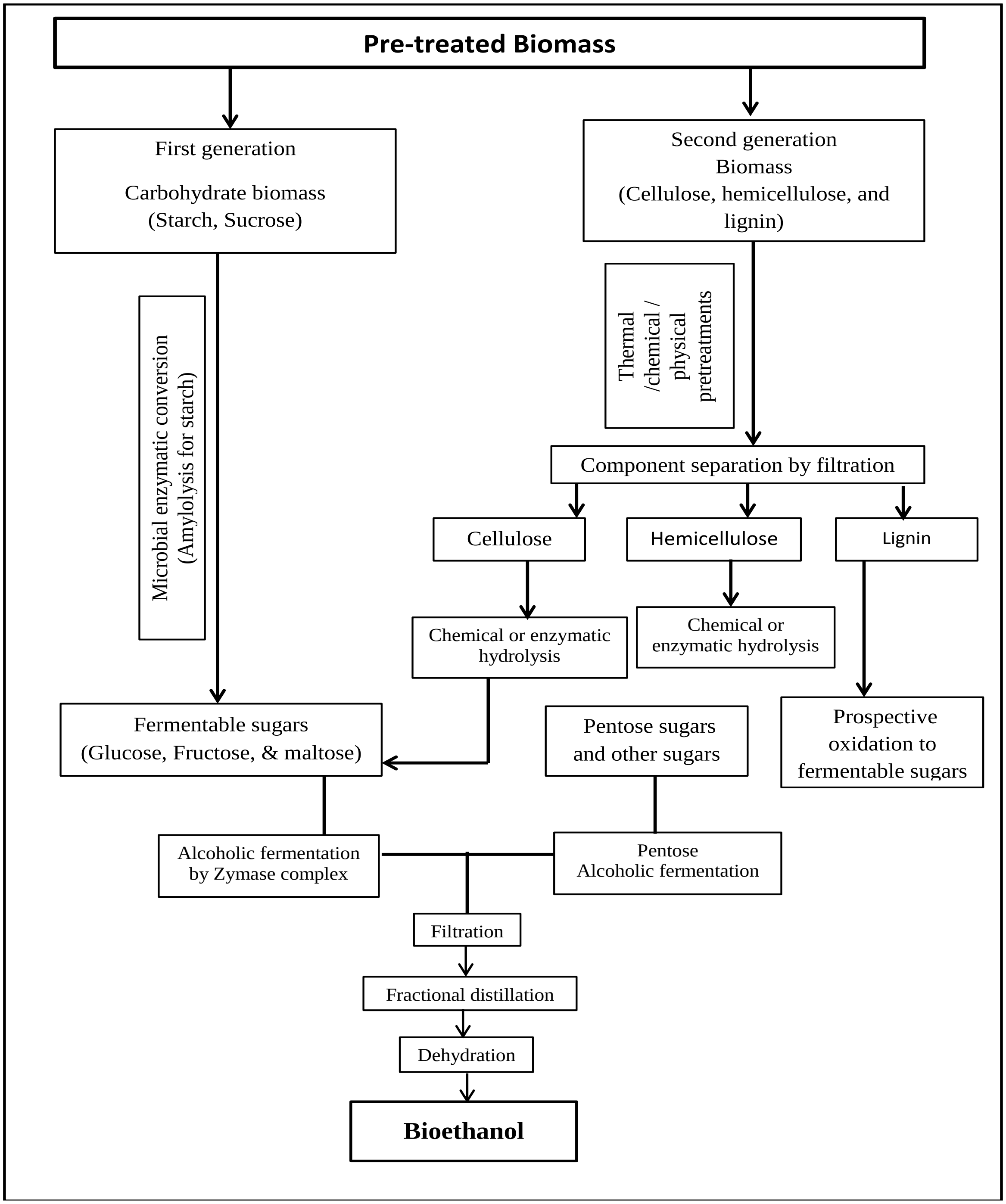

Figure 1.2. Flow chart for first and second generation bioethanol production (Zammit, 2013).

In 2011, biodiesel consumption was 1.5% of distillate fuel (e.g. diesel) by volume while bioethanol consumption was 9.6% of gasoline by volume (EIA, 2012).

Biofuels not only serve as an energy source, but its usage can also reduce environmental pollution. Emission from biodiesel contains 12, 21, and 12% less particulate matter, hydrocarbons, and carbon monoxide, respectively, when used in B20 grade fuel (20% blend of biodiesel) (EPA, 2001). The use of bioethanol reduces 86% greenhouse gas (GHG) emission compared to gasoline (Wang et al., 2007). Wang et al. (2007) also found sugarcane biomass produced about four times lower GHG emission than corn (*Zea mays*). However, conflicts still remain among scientists regarding the effect of biofuel production and consumption on environmental quality. Some researchers reported that due to increasing demand for biofuel crops, natural forests and pastures are being converted to agricultural land, and the carbon stored as above ground and underground biomass of plants is converted to CO_2 during microbial decomposition (Fargione et al., 2008). Crops, such as poplar (*Populus spp.*), willow (*Salix spp.*), eucalyptus (*Eucalyptus spp*.), emit large amounts of isoprene which react with ozone and form chemical compound responsible for haze (Reuters, 2013). In contrast, biofuels on average can reduce GHG emissions by 30-50%. Thus over time, the use of biofuels as a primary energy source can offset its adverse effects on land-use changes. Bioethanol contains 35% more oxygen which facilitates complete combustion of fuel and reduction in the amount of harmful components of vehicle emissions (RFA, 2005). Also, it does not include carcinogenic chemicals like benzene, toluene, etc.

The United States (U.S.) is the second largest producer of biodiesel (after European Union) and the largest producer of bioethanol in the world. Most of bioethanol is produced from corn grain (Dien et al., 2002; Duval et al., 2013), and production has increased by 42% from 2007 to 2011 (EIA, 2012). Because of the increasing demand for corn-based bioethanol production, corn grain price was increased by 48% within five years (USDA–ERS, 2011).

Therefore, due to several drawbacks of grain crops as a source of biofuel (i.e., continuous increase of food grain price, higher management cost, and decrease of biodiversity), the use of cellulosic biofuels has gained more interest (IEA, 2008). Cellulosic biofuels can be produced from non-food agricultural and forest residues and different biofuel crops which can be grown in marginal or degraded land with minimum inputs.

1.3 Energy cane as a biofuel crop

Some of the cellulosic biofuel crops found in the U.S. are energy cane (interspecific *Saccharum* hybrids), elephant grass (*Pennisetum purpureum*), switch grass (*Panicum virgatum*), miscanthus (*Miscanthus*×*giganteus*), corn stover, sweet sorghum (*Sorghum bicolor*), giant reed (*Arundo donax*), canary grass (*Phalaris canariensis*), and cottonwood (*Populus fremontii*). However, among these different potential biofuel crops, energy cane can be very promising for development of a biofuels industry in Louisiana because of warm temperatures, long growing season (230 to 290 days) and high annual rainfall (163 mm) (Kim and Day, 2011). Energy cane derived from the hybridization of sugarcane (*Saccharum officinarum*) and wild cane (*Saccharum spontaneum*) increases biomass production potential of sugarcane (Flora of North America Editorial Committee, 1993; Viator and Richard Jr., 2012). Sugarcane bagasse contains 42% cellulose, 25% hemicelluloses, and 20% lignin, while energy cane bagasse contains 43% cellulose, 24% hemicelluloses and 22% lignin (Kim and Day, 2011). Also, energy cane juice contains 10% fermentable sugar. The sugar or starch, cellulose, and hemicellulose derived from energy cane can be used for ethanol production and that bioethanol later on serves as a source of fuel for vehicles in its pure form, but generally is used as a gasoline additive to increase octane content and to improve vehicle emissions.

1.4 Importance of crop yield estimation

Energy cane is a C4 plant and can produce a large amount of dry matter throughout the growing season (Turhollow et al., 1990; Lewandowski et al., 2003) Studies reported that in Florida, energy cane (US 72-l153) produced an average of 48 Mg dry biomass ha^{-1} yr^{-1} within a four-year growing period with a nearly 53% yield reduction from plant cane to the third ratoon crop (Mislevy et al., 1995). In general, nutrient uptake is positively correlated with crop yield. On average, 1 Mg plant residue may contain 20 kg of nitrogen (N), 2 kg of phosphorous, and 15 kg of potassium as primary nutrients, with 80 kg of calcium, 20 kg of magnesium, 10 kg of sulfur as secondary nutrients, and 0.1 kg of iron, 0.01 kg of manganese, 0.04 kg of zinc, 0.03 kg of boron, 0.005 kg of copper, 0.001 kg of molybdenum as micronutrients (Coombs et al., 1996). Hence, modern high yielding crops like energy cane not only provide higher production of biomass, but also remove a proportional amount of nutrient from the soil. Nitrogen is very dynamic in nature and very difficult to manage due to its wide oxidation state (-3 to +5). So application of N should be very precise to improve crop uptake and reduce the amount of loss from the soil profile. If a relationship between the amounts of N removal per unit of biomass produced can be established, then it will be easier to calculate the N requirement of a plant.

Post-harvest loss of sucrose content in cane is very high due to inversion of sugar. In a study conducted in northern part of India by Kulkarni and Warne (2004), that the magnitude of post-harvest loss was as high as 25 kg per ton of cane. For burned cane, the loss is 2-3 times higher than the unburned cane. Sugar present in cane gets converted into dextran a polysaccharide which degrades sugar quality by blocking filtration and crystallization of sugar in the processing unit over time and cause difficulty for processing (Bakker, 1999). The production of dextran is positively correlated with the time gap between harvesting and milling of cane

(Bakker, 1999). Therefore, if the yield at harvest can be measured, then planning of staggered harvesting can also be done depending on the availability of the processing unit to prevent the quality deterioration of cane.

Yield estimation is very important for crop insurance to cope up with the risk factor related to agricultural crop production because of its high dependence with environmental conditions. If there is crop damage due to hail storm, for insured farms, farmers will get paid depending upon the quantity as well as quality of loss. Estimation of yield also provides information of income and expenses ahead of time to make a budget. On a large scale, it is important to measure yield by remote sensing for a nation to have an idea about production of crop which affects the decisions related to the export and import of foods and feed (Shafian and Valadanzouj, 2007). If the estimated yield indicates surplus of crop production, then the excess amount can be exported while if it indicates shortage of production, then import of foods can be done.

1.5 Different procedures for crop yield estimation

Crop yield estimation can be done through variety of ways. In general, destructive biomass sampling is the most accurate and direct method to measure yield of a plant. For some plants, it has been reported that the best way to predict biomass productivity is to sample above ground biomass sequentially throughout its growing period (Odum, 1960; Ovington et al., 1963; Singh and Yadava, 1974; Vina et al., 2004). The basic method of yield determination is called plot yield method where one square meter area is selected randomly using a quadrant (a rectangle frame) and then the yield obtained from that area is measured and converted to kg or t ha^{-1}. In a plot yield method, a random subplot is usually selected with the quadrant (Spencer, 1989; Fermont and Benson, 2011). Harvesting is done leaving the plants in the quadrant area.

After finishing the whole plot, the quadrant is harvested and measured. Sometimes one quadrant is not enough for the entire field because it is too small to represent its variability in terms of production. Hence, two large quadrants (50-75 m^2 each) (Fielding and Riley, 1997; Fermont and Benson, 2011) or multiple quadrants (Norman et al., 1995; Fermont and Benson, 2011) are used. Estimation of yield can also be done from the long term yield potential of that area. To estimate crop yield depending on the available historical data, a better understanding of the trend of weather, cultivars and soil type is needed. Crop yield estimation can also be done by remote sensing. Remote sensing is defined as "the science and art of obtaining information about an object, area, or phenomenon through the analysis of data acquired by a device that is not in contact with the object, area, or phenomenon under investigation" (Lillesand et al., 2008). Plants contain pigments (e.g. chlorophyll, carotenoids) which absorb more light in blue and red wave band and reflect higher in the near infrared (NIR) wave band (Daughtry et al., 2000). If the plant is stressed, it starts losing chlorophyll and reflects more in visible, i.e. red and blue wave bands. This is the principle behind the usage of remote sensing to estimate crop growth vigor and yield potential.

1.6 Remote sensing and crop yield estimation

Among different yield determining procedures, the use of sensors is gaining popularity because it provides real-time information as well as being nondestructive. In addition, it is faster than the conventional method of yield estimation and applicable for larger area. Remote sensing for yield estimation can be done by satellite sensors, air-borne sensors, and ground-based sensors (Li, 2010; Nuarsa, 2011; Sakamoto, 2013; Geipel, 2014). The ground-based sensors can be hand-held, tripod, or crane mounted. In air-borne remote sensing, sensors are mounted in an airplane, balloon, helicopter, or unmanned airborne vehicles (UAV). Space borne sensors are mounted in a

satellite that are: geostationary (situated above equator and rotating along with the earth in the same pace and as a result it looks like stationary e.g. GOES-15 and GOES-13) and polar orbiting or sun synchronous (rotating from pole to pole of the earth at 90^{o} angle with the equator). It is traveling from North to South Pole but crossing a particular point on earth at a particular time (e.g. LANDSAT).

Depending on the light source, sensors are of two types: active and passive sensors. Active sensors have their own source of light and can work independently irrespective of the presence of sun whereas, passive sensors do not have any light source and dependent on solar radiation or other artificial source of illumination (Aggarwal, 2004). As explained earlier, chlorophyll has great influence on plant canopy reflection characteristics. Remote sensing technology uses the reflection property of the plant to calculate vegetation indices (VI) and estimate yield potential (Unganai and Kogan, 1998; Labus et al., 2002; Prasad et al., 2006; Ren et al., 2008). Vegetation indices are determined with mathematical calculation using two or more wavelengths reflected from vegetation surfaces and provide some information about it. Calculation of VI helps to reduce the background noise (e.g. reflection from soil, scattering by dust particles, water vapor, reflection coming from unwanted area, and water background) using more than one wavelength for calculation as all wavelengths are affected similarly (Huete et al., 1985; Zhu et al., 2008). Different VIs are calculated in different ways to get different information about any particular vegetation. Vegetation indices can be used successfully to measure different crop parameters such as leaf area index (LAI) (Gong et al., 2003), crop cover (Purevdorj et al., 1998), moisture stress (Ceccato et al., 2001), stress due to plant pathogens (Genc et al., 2008), yield potential estimation (Panda et al., 2010) and N application rate (Raun et al., 2002). So, with

the help of crop growth monitoring over time, we can optimize natural resources and pest control measures to maximize crop yield.

Watson (1947) first used LAI which is the ratio between total leaves areas present per unit ground surface areas. Leaf area index is related to the amount of light intercepted by plant canopy. If the LAI value is lower than 1, then it allows higher solar light to pass through the canopy and then to soil. Higher LAI means denser canopy, more light energy interception, less light energy passing to the soil and higher production efficiency of crops (Wolf et al., 1972). According to Gong et al. (2003) forest LAI has a high correlation with shortwave infrared (SWIR) and some NIR wavebands as these wavebands are affected by moisture, and biochemical content (lignin, protein, cellulose, sugar, and starch) of the plant. High density vegetation cover can be estimated using normalized difference vegetation index (NDVI), simple ratio (SR) (Gamon et al., 1995; Dobrowski et al., 2002), and perpendicular vegetation index (PVI) (Dobrowski et al., 2002), while low density can be better estimated by soil adjusted vegetation index (SAVI) (Purevdorj et al., 1998). According to Huete (1988), NDVI and PVI are highly dependent on soil reflection and any adjustment worked better for the accuracy of the estimation. Also, SAVI with correction factor L=0.5 performed better to reduce soil noise for both broadleaf and grassy canopy crops. If the soil line (linear relationship of red and NIR reflectance of bare soil, Bareta et al., 1993) is known, then vegetation cover can also be accurately estimated by transformed SAVI (TSAVI) (Purevdorj et al., 1998). According to Raun et al. (2002), in-season estimated yield (INSEY) and NDVI-estimated N response index (RI) were useful to variably apply N to crop on a need-basis; this N application method provided an average of 187 kg ha^{-1} higher grain yield than the mid-season fixed N rate (45 kg N ha^{-1}). Generally, INSEY is calculated by dividing NDVI reading with growing degree days. Response index calculated at

early crop stage can be a useful tool to predict N yield response at harvest (Mullen et al., 2003). Response index at mid-season (RI_{NDVI}) is the ratio between averages NDVI from N rich strip to NDVI from control plot. Vegetation index can also perform as an efficient tool for determining leaf N content (Zhu et al., 2008).

For determining vegetation water content, SWIR can be used but SWIR alone cannot measure plant water content as it is also affected by two other plant parameters i.e. leaf internal structure and biomass production. On the other hand, NIR is also affected by leaf structure and biomass production. So, VI, that is calculated using SWIR and NIR, can predict leaf moisture content accurately (Ceccato et al., 2001). According to Genc et al. (2008), the level of disease infection in plant population can be determined by using remote sensing. Pest damage affects crop externally as well as internally. External changes are leaf drop, necrosis, and withering of foliage while the internal factors are destruction of chlorophyll and leaf color change which affects light reflection pattern from the plant canopy (Ren et al., 2010). Affected plants reflect a higher amount of light in the visible region due to destruction of foliage, chlorophyll and less amounts of light in NIR region due to destruction or alteration in leaf internal structure (Hatfield and Pinter, 1993). Thus VI computed from reflectance reading of NIR and visible wavelengths can be used to estimate level of pest infestation in plants. However, readings of pest infestation and nutrient stress produce similar symptoms and canopy spectral reflectance pattern. Thermal infrared (TIR) can be used to measure diseases that affect water movement inside the plant. Sugar beet leaf affected by *Pythium aphaniderrnatum* is 3^{o} warmer than healthy plants (Pinter et al., 1979; Hatfield and Pinter, 1993). Therefore, application of TIR for the measurement of plant diseases is also affected by physical stress like salinity and drought (Hatfield and Pinter, 1993).

Thus, practical application of remote sensing is very difficult for accurate detection of plant disease (Huang, 2012).

In remote sensing the commonly used VI for measuring different crop parameters are SR, NDVI, $NDVI_{red\text{-}edge}$, $SR_{red\text{-}edge}$, enhanced vegetation index (EVI), SAVI, PVI, difference vegetation index (DVI), and weighted difference vegetation index (WDVI).

1.7 Characters of leaves that affect canopy spectral reflection

Solar radiation is the main source of light for the earth. Solar radiation that falls on a plant canopy can be reflected, absorbed, and transmitted. According to Nobel (1991) a single leaf can reflect 6%, absorb 90%, and transmits 4% of the photosynthetic photon flux. Photon flux can be described as the moles of photon (400-700 nm) per unit area at per unit of time (Bugbee and Monjee, 1992). Leaf reflection plays the most important role in remote sensing. It depends on leaf thickness, presence of wax, leaf pigments, leaf water content, leaf chemical composition, leaf canopy structure, and plant stress. The cuticle, a protective cover on the leaf surface, acts as a barrier for photon penetration and absorption by leaves (Yang et al., 2013) and it reflects more in NIR wavelength (800 nm) (Slaton et al., 2001). Leaf hairs increase the amount of reflection from the surface and act as a reflector. According to Ehleringer et al. (1976), leaf hairs present in *Encelia ferinosa* reduces absorption of sunlight in the visible range (400-700 nm) by 56% from its non-pubescent relative (*Encelia californica*) and as 1% is transmitted through leaves, and the rest of the light is reflected due to leaf hairs. Slaton et al. (2001) reported that NIR reflection directly related with leaf bicoloration (leaf lower surface is lighter in color than upper leaf surface), amount of cuticle present, and amount of mesophyll cells exposed to intercellular air space per unit leaf area. Reicosky and Hanover (1978) reported that the presence of wax on the leaf increases leaf reflectance. Glaucous (bluish, grayish and whitish wax coating on leaf that can

be removed by gentle rubbing) leaves reflected more light in range of 350-800 nm than non-glaucous leaves of blue spruce (*Picea pungens*), and the reflectance gradually decreased from UV and blue wavelength towards 800 nm. Canopy reflectance is also affected by the pigments present in the leaf. Under stress condition or during fall color (i.e. low temperatures and shorter day length), disintegration of chlorophyll occurs earlier than carotenoid which gives foliage a yellow, orange color (Ustin et al., 2009). During leaf senescence or at an early stage (before establishment of photosystem), anthocyanin present in the leaves provides a red color. According to Lal et al. (1952), green pigments disintegrate at a higher rate than yellow pigments, and disintegration of green pigments starts after third leaf stage while disintegration of yellow pigments starts after sixth leaf stage in sugarcane. Carotene has absorption peak at 400–500 nm and reflectance in the red region (Sims and Gamon, 2002). Anthocyanin absorption band is 550 nm (Sims and Gamon, 2002). Absorption in SWIR in 1350-2500 nm is due to water present in leaves (Slaton et al., 2001). Thermal infrared (6.0-15.0 µm) provides information about plant evapotranspiration (Ceccato et al., 2001). Foliar reflectance can also be influenced by chemical content like N, lignin, and cellulose. Cellulose and lignin has good correlation in mid infrared range of wavelength after the water absorption peak as well as in NIR (Jacquemoud et al., 1996). Lignin has a strong absorption band at UV range i.e. 280 nm and continued until NIR (Dawson et al., 1998).

1.8 Effect of plant pigments and cellular structure on canopy spectral reflectance

Generally, crops absorb more light in the visible range due to the presence of chlorophyll and reflect NIR range due to the internal scattering by leaves. Blue absorption peak cannot be used for the study of chlorophyll as it overlaps with the carotenoids absorption peak (Sims and Gamon, 2002). Light is generally scattered and reflected back while traveling from a medium

with higher refractive index (1.4) i.e., plant cells to a medium with lower refractive index (1.0) i.e., air. Hence, the plant cell-air interface plays a great role for NIR reflection. Plant leaf structure varies greatly between species and environmental conditions. Generally, plant leaves contain have two kinds of cells: palisade parenchyma (elongated cells at the upper side of the leaf), and spongy parenchyma (spherical shape cells at the lower side) wherein chlorophyll is largely present in palisade parenchyma cells (Gates et al., 1965).

Other pigments such as carotenoids, xanthophylls, and anthocyanin also have large influence on photosynthesis. Carotenoid and xanthophyll are known as accessory pigments. The light energy harvested by these pigments is sent to chlorophyll (Majumdar, 2011). When the energy of solar radiation exceeds the energy requirement for photosynthesis, carotene dissipates the excess energy through a xanthophyll cycle (Sims and Gamon, 2002). In plants there are three carotenoids, violaxanthin, antheraxanthin, and diadinoxanthin that take part in the xanthophyll cycle and de-epoxidised removal of epoxy group to diatoxanthin and zeaxanthin. During light stress violaxanthin get converted to zeaxanthin through an intermediate product antheraxanthin in lumen side with the help of violaxanthin de-epoxidase enzyme (Yamato et al., 1962; Matsubara et al., 2003). The excess energy then dissipates through heat energy by the antennae complexes of photosystem II. Zeaxanthin produces violazanthin in stroma in the presence of zeaxanthin epoxidase enzyme either in dark or low light condition (Matsubara et al., 2003).

$$\text{Violaxanthine} \xrightarrow{\text{violaxanthin de}-\text{epoxidase}} \text{Antheraxanthin} \xrightarrow{\text{violaxanthin de}-\text{epoxidase}} \text{Zeaxanthin}$$

$$\text{Zeaxanthin} \xrightarrow{zeaxanthin\ epoxidase} \text{Antheraxanthin} \xrightarrow{\text{zeaxanthin epoxidase}} \text{Violaxanthin}$$

Violaxanthin has an absorption peak in the ultraviolet region 440–470 nm ((Jeffrey et al., 1997). Chlorophyll has high absorption in red 660-680 nm and high reflection in 550 and 700 nm

(Sims and Gamon, 2002). Carotenoid has absorption in the wavelength range 400–500 nm with high reflectance in red (Sims and Gamon, 2002). Lutein absorbs light at 495, 466, and 437 nm while neoxanthin absorbs at wavelength 486, 457, and 430 nm (Ruban et al., 2000). Antheraxanthin has a maximum absorption band at 447 nm (in ethanol) (Ruban et al., 1993) and β– carotene has an absorption band at 455 nm (when the solvent is acetone) (Zsila et al., 2001). Chlorophyll is the main photosynthetic pigment of plant and helps to absorb solar radiation and convert it to chemical energy during photosynthesis. Chlorophyll 'a' has an absorption peak at 700 and chlorophyll 'b' has an absorption peak at 680 nm and known as P700 (present in PS I) and P680 (present in PS II) (Ishikita et al., 2006). During light reaction, ATP and NADPH are produced which are responsible for fixation of carbon dioxide in the absence of light (Mauseth, 2011).

Anthocyanin mostly present in lower epidermal cells (Sims and Gamon, 2002) also protects the photosynthetic system from excess solar radiation (Gould et al., 1995; Barker et al., 1997; Dodd et al., 1998; Sims and Gamon, 2002) and ultra violet (UV) radiation (Burger and Edwards, 1996; Klaper et al., 1996; Mendez et al., 1999; Sims and Gamon, 2002). Anthocyanin also helps to scavenge free oxygen radical in plant cells. Proanthocyanidins and anthocyanins mainly accumulated in vacuoles and protect plant cells from reactive oxygen species (ROS). The formation of proanthocyanidins from Flavan-3-ols requires oxidation which might utilize hydrogen peroxide or ROS and thus neutralize its harmful effect (Hernández et al., 2009).

1.9 Cane planting materials

Canes are predominantly planted as whole stalk and billets with their own benefits. Generally, billets are produced by chopping cane stalks with the use of combine harvester (Hoy, 2001). According to Salassi et al. (2004) hand and machine-planted whole stalk required 7.5

times and 5.5 times lower planting materials than the billet-planted cane. As billets are shorter than the plant cane and the cumulative amount of exposed surface is higher than the whole stalks, it can facilitate disease infestation like red rot (On splitting of cane stalk, internal part shows red discoloration with acidic smell due to degradation of sucrose) (Yin et al., 1996; Hoy, 2001; Hoy et al., 2004). Combine harvester or chopper is better for lodged cane than the whole stalk harvester (Hoy, 2001). Uneven crop stand in whole stalk planted cane was observed due to low germination in lower buds of the stalk caused by apical dominance. This is the effect of auxin hormone which suppresses the germination of the lower buds and promotes the apical bud (Orgeron et al., 2007).

1.10 Rationale

There are different established algorithms for yield potential estimation for different crops for different regions. The most common and widely used procedure for yield estimation is to correlate the early-season canopy spectral reflectance with yield at harvest (Teal et al., 2006). Algorithms have been established for corn (Prasad et al., 2006), soybean (Prasad et al., 2006), and wheat (Raun et al., 2005) based on this principle, but a very limited amount of work has been done to evaluate the pattern of relationship between early-season canopy spectral reflectance and biomass yield collected at the same time.

Cane planting materials commonly include billets and whole stalks. Planting materials affect the survival rate and establishment of cane. For a plot with uneven cane population stand, the sensor sense soil as well as plant. Due to unevenness of the distribution of crop canopy, variability among NDVI readings will be larger which leads to higher CV value and vice-versa. Because of better canopy structure in those plots, the pooled NDVI might be higher which may generate higher N application rate for that plot, but the rate application should not be even for all

the patches of crop stand. According to Arnall et al. (2006) plots with higher CV value (>26) cannot respond to the top dressing application of N fertilizer. Hence incorporation of CV among VI in yield estimation algorithms can be a powerful tool for N recommendation for site-specific nutrient management.

Therefore, this current study was established to fill the gap between the existing information and establish the relationship between early-season canopy spectral reflectance (within the visible and near-infrared wavebands) and the biomass yield obtained at the same time as well as the N relation between the early season agronomic parameters with the parameters obtained at harvest. Additionally, this study was design to determine if CV among NDVI readings can be used to monitor cane population stands.

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Chapter 2. Use and Application of Spectral Vegetation Indices to Relate Early-Season Biomass Yield of Energy Cane

2.1 Introduction

Determination of vegetation indices (VI) involves mathematical computation of different spectral reflectance collected from plant canopy and can provide some information regarding plant properties (Ray, 1994). The VIs are of two types: ratio VI and perpendicular VI (Jackson and Huete, 1991). For remote sensing, mostly red and near infrared (NIR) wavelengths are used for VI calculation. Hence, red-NIR soil line is calculated from bare soil by plotting red at x-axis and NIR at y-axis. According to Ray (1994), lines of identical vegetation may converge in a single point. The slope of the line connecting the convergent point and red-NIR point is calculated for ratio vegetation index e.g. SR, NDVI, and SAVI. In contrast, the lines of identical vegetation may be parallel to soil line and the perpendicular distance between them is measured for perpendicular indices e.g. PVI, DVI, and WDVI.

Different vegetation indices like simple ratio (SR), normalized difference vegetation index (NDVI), $NDVI_{red\text{-}edge}$, $SR_{red\text{-}edge}$, and modified red-edge SR are commonly used to predict biomass yield (Hansen and Schjoerring, 2003; Mutanga and Skidmore, 2004; Vina and Gitelson, 2005; Grant et al., 2012).

According to Taylor (1997), health of the crop can be determined using the amount of light absorption by plant canopy in red wavebands and reflectance in NIR wavebands. The canopy reflectance for calculation of VI can be collected from the canopy of plants in experimental plots as well as from single leaves in the laboratory with artificial source of light. The single leaves always provide higher signal to noise ratio than the plant canopy (Ritchie, 2003).

Todd et al. (1998) used different spectral indices to estimate biomass on grazed and ungrazed rangelands. They used green vegetation index (GVI), brightness index (BI), wetness index (WI), NDVI, and the red waveband (RED). They found the RED was more sensitive than NDVI and GVI for estimating biomass in grazed as well as ungrazed sites. Panda et al. (2010) used a SLR 35 mm camera from an airplane to obtain images of corn (*Zea mays*) field in Oakes irrigation test area, North Dakota and calculated PVI, NDVI, GVI, and SAVI for corn yield estimation before harvest. They found PVI accomplished the objective better than the others as it reduced bare soil interference. Joanes et al. (2004) used an ultrasonic sensor to measure plant height and multispectral image data to measure the crop coverage. The product of these two provided good correlation with biomass in corn and spinach (*Spinacia oleracea*) but poor correlation for snap beans (*Phaseolus vulgaris*). Muskova et al. (2008) worked on the application of NDVI for measurement of biomass of differently managed mountain meadows (mown, mulched, and unmanaged). The study showed the relation between biomass yield and NDVI was significantly affected by different treatments. Normalized difference vegetation index showed high correlation with biomass production up to peak growth stage but after mowing and mulching, the relationship become non-significant. In unmanaged condition, NDVI showed high correlation with biomass production for all growth stages. Mutanga et al. (2012) had a similar finding on WorldView-2 satellite imagery for NDVI measurement to predict yield of wetland biomass. Conventional NDVI could predict the high density biomass yield of wetland vegetation but the predictability was increased with incorporation of red-edge (centered at 725 nm) and NIR in calculation of NDVI.

There have been reports regarding some drawbacks of using red wavelength in calculating VI in high biomass producing crops. Chlorophyll has a strong absorption in red

wavelength 660-680 nm and after certain concentration of chlorophyll a, red light loses its sensitivity (Wu, 2008; Yang, 2008; Zhaoa et al., 2012). It is well-documented that NDVI get saturated at plant canopy closure and become insensitive to further development of crops (Sellers, 1985; Baret and Guyot 1991; Buschmann and Nagel, 1993; Gitelson et al., 2002a, 2002b, 2003). The use of red-edge and NIR can predict yield more accurately and not get saturated very fast (Mutanga and Skidmore, 2004a; Chen et al., 2009a; Mutanga et al., 2012). Narrow band-derived VI was used to estimate yield of high biomass producing crops. Energy cane (interspecific *Saccharum* hybrid) produces a very high amount of biomass and thus it is imperative to identify VI which has the ability to discriminate energy cane yield biomass without getting saturated even at canopy closure.

The numerous benefits associated with crop yield estimation have stimulated research interest in this field. Vegetation indices have been used extensively to maximize the yield prediction level. Plants are very different in nature, and it is difficult to fit all of the crops under same algorithm. That has led to varied responses using different VI on different crops. Therefore, the overall objective of this study was to evaluate the feasibility of using spectral reflectance-based VI for non-destructive estimation of energy cane total biomass yield.

2.2 Specific Objectives:

1. To evaluate the feasibility of using spectral reflectance-based vegetation indices to estimate energy cane biomass yield
2. To identify the most sensitive wavelength to correlate early-season crop biomass production
3. To compare the relation between early-season biomass yield and different vegetation indices in energy cane; and

4. To determine the most appropriate time frame for sensing to predict yield

2.3 Materials and Methods

2.3.1 Site location and characteristics

The experiment was established at the Louisiana State University Agricultural Center Sugar Research Station in St. Gabriel at two sites: A (30º15'47"N 91º05'54"W) and B (30º16'08"N 91º06'10"W). Site A was planted in 2012 and site B was planted in 2013. Hence, this research experiment collected data from two plant cane and one first ratoon crops. Before planting, four composite soil samples with 20 cores for each quadrant were taken from both experimental sites at 15 cm depth with an auger. Site A soil was acidic (pH = 5.5) with a C:N ratio 6:1, while site B was slightly acidic (pH = 6.04) with a C:N ratio of 7:1. Multi-element analysis based on Mehlich-3 procedure (Mehlich, 1984) was done for both background soil samples (Table 2.1). The soil type was Commerce silt loam (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent).

Table 2.1. Chemical properties of composite soil samples collected from site A and B.

Properties	**A**	**B**	**Interpretation**
Soil type/texture	Commerce silt loam		
Soil classification	fine-silty, mixed, nonacid, thermic Aeric Fluvaquent		
pH	5.5	6.04	
C:N ratio	6:1	7:1	
Phosphorus, mg kg^{-1}	34	41	High
Potassium, mg kg^{-1}	170	219	High
Magnesium, mg kg^{-1}	458	572	Very high
Sulfur, mg kg^{-1}	10.8	11.7	Medium
Copper, mg kg^{-1}	3.5	4.7	Within critical limit

2.3.2 Layout of the experimental plot

Total size of the experimental area for site A was 1572 m^2. There were three rows in each plot, and the row length was 9.1 m. Spacing between rows was 1.8 m. The length of the alley between plots was 3 m. For site B, total size of experimental area was 1317 m^2. Each plot consisted of three 7.62 m long rows with 1.8 m spacing between rows with a 1.5 m alley. For both sites, treatments were arranged in a split-plot with randomized complete block design (RCBD) where two energy cane varieties, Ho 02-113 and US 72-114 were designated as the main plot, and N rates (0, 56, 112, and 224 kg ha^{-1}) as the sub-plot treatments with four replications.

2.3.3 Planting and fertilizer application

Crop physiology of energy cane is very similar to sugarcane, and it can be planted using whole stalks or stalk sections (billets). In the current study, energy cane was planted using billets on September 17, 2012 and October 14, 2013 for sites A and B, respectively. The billets were cut with a combine harvester in the range of 50-55 cm in length with approximately three buds per billet. Elevated beds were opened; and five to six running billets were placed in the planting furrow. Fertilizer N was applied in April at the rates of 0, 56, 112, and 224 kg ha^{-1} in the form of urea ammonium nitrate (UAN, 32% N) using a UAN applicator. The amount of K was 60-80 kg ha^{-1} and no P was applied.

2.3.4 Vegetation indices

(a) Simple ratio (SR): It is one of the old spectral vegetation indices. It is computed using the following formula (Jordan, 1969; Ritchie, 2003):

$$SR = \frac{\rho_{NIR}}{\rho_{red}}$$

Where ρ = reflectance.

Simple ratio is the ratio between the reflectance at the near infrared (NIR) and red bands, and the value of SR ranges from 1 to 30. For bare soil, where the reflection is nearly similar in red and NIR bands, the value tends to be 1. As the greenness or chlorophyll content of plants increases, the ratio increases. Sometimes at high plant density, the red wave band reflection is very low or tends to zero, and SR calculation will increase. Hence, for better response, red reflection should be measured very carefully and precisely (Jackson and Huete, 1991).

(b) Normalized Difference Vegetation Index (NDVI): Normalized difference vegetation index (Rouse et al., 1973) was computed as:

$$\mathrm{NDVI} = \frac{\rho_{\mathrm{NIR}} - \rho_{\mathrm{red}}}{\rho_{\mathrm{NIR}} + \rho_{\mathrm{red}}}$$

The value of NDVI generally ranges from -1 to +1 and specifically ranges from 0.2 to 0.8 for normal vegetation, negative values for water and around 0 for bare soil. Healthy plants with dense canopy give an NDVI value near 1.

(c) Red-Edge Normalized Difference Vegetation Index ($\mathrm{NDVI}_{\text{red-edge}}$): For this modification of NDVI, measurements were obtained using reflectance at NIR and red-edge region. Figure 2.1 shows where the reflectance measurements for the REP (red-edge position) are obtained.

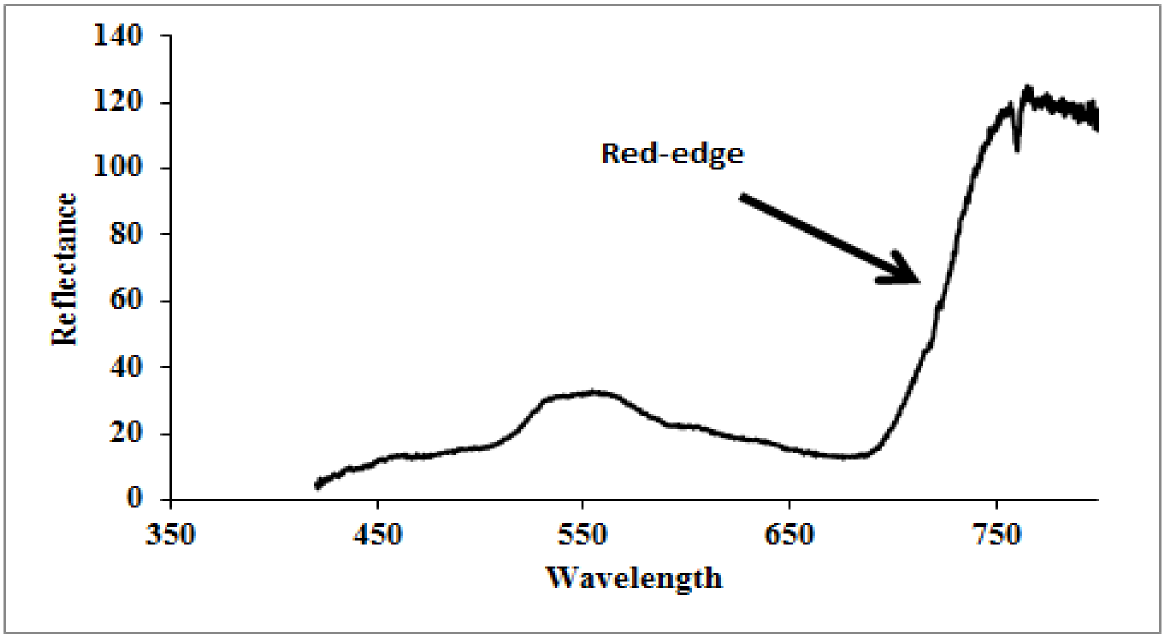

Figure 2.1. Graphical representations of red-edge position.

Red-edge is the sharp increase of reflection between the red wavebands to the NIR wavebands. Earlier studies showed that the reflectance reading at the REP is related to plant chlorophyll content (Horler et al., 1983; Clevers et al., 2001). The computational formula of $NDVI_{red\text{-}edge}$ is:

$$NDVI_{red-edge} = \frac{\rho_{NIR} - \rho_{red-edge}}{\rho_{NIR} + \rho_{red-edge}}$$ (Sims and Gamon, 2002)

(d) Red-edge simple ratio index: This is a modification of the SR index which uses red-edge reflectance. The formula is:

$$SR_{red-edge} = \frac{\rho_{NIR}}{\rho_{red-edge}}$$ (Sims and Gamon, 2002)

Red-edge position can be determined using the following methods:

1) Linear interpolation: Detection of REP can be simplified by a straight line centered at the middle point between highest reflectance wavelengths (780 nm) to lowest reflection wavelength (670 nm). In this case, calculation of inflexion point is done first. Then a linear interpolation between 700 and 740 nm is done to estimate the waveband corresponding to the inflexion point (Guyot et al., 1992; Danson and Plummer, 1995; Dawson and Curran, 1997). It was calculated using following two steps (Cho et al., 2008):

Calculation of reflectance at inflexion point (ρ_e):

$$\rho_e = \frac{\rho_{670} + \rho_{780}}{2}$$

Calculation of REP:

$$REP = 700 + 40\left(\frac{\rho_e - \rho_{700}}{\rho_{740} - \rho_{700}}\right)$$

Where 700 = constant

40 = constant and obtained from interpolation between 740 and 700 nm.

2) Maximum first derivative: In this case, REP is the maximum first derivative of reflectance in the red-edge region (Dawson and Curran, 1998; Cho et al., 2008). It is calculated as:

$$FD\rho_{\lambda i} = \frac{\left(\rho_{\lambda(j+1)} - \rho_{\lambda(j)}\right)}{\Delta_\lambda}$$

FDρ= first derivative reflectance at a wavelength i midpoint between wavebands j and j+1

Rλ(j) = reflectance at the j waveband

Rλ(j+1) = reflectance at the j+1 waveband

Δλ = difference in wavelengths between j and j+1.

3) Linear extrapolation technique: Linear extrapolation technique is used to mitigate the double peak problem due to the correlation between REP and chlorophyll content and track changes in slope near 700 nm and 725 nm (Cho and Skidmore, 2006; Cho et al., 2008). In this method, the REP is situated at the intersection between far-red line and NIR line. It can be calculated as:

First derivative reflectance of far red line: $FD\rho \ = \ m_1\lambda + \ c_1$

First derivative reflectance of NIR line: $FD\rho \ = \ m_2\lambda + \ c_2$

Hence, $REP = \frac{-(c_1 - c_2)}{(m_1 - m_2)}$

4) Polynomial fitting technique: Polynomial fitting technique was done to the reflectance obtained from wavelengths 670 nm to 780 nm, respectively (Pu et al., 2003, Cho et al., 2008). In this case third polynomial fitting was done (Clevers and Buker,1991). Third order polynomial fitting model can be calculated as:

$$\rho(\lambda) = \ a_{0+\sum_{i=1}^{3} a_i \lambda^i}$$

Then, REP was determined by maximum first derivation of its functions.

According to Clevers and Buker (1991), the problem of polynomial fitting technique is its symmetry around the REP.

5) Lagrangian technique: It is a three point Lagrangian interpolation technique for REP calculation (Dawson and Curran, 1998). It is determined by the first derivation of the reflection. Here λ_i is the waveband having maximum first derivative. λ_{i+1}, λ_{i-1} is the waveband situated on both side of λ_i. Additionally, $D_{\lambda(i)}$, $D_{\lambda(i+1)}$, and $D_{(\lambda i-1)}$ are the first derivative values for the respective wavebands. This technique used second order polynomial to fit the first derivatives wavelengths which has center at maximum first derivative (Pu et al., 2003). To identify the wavelength having maximum slope, second derivation was then performed on Lagrangian

interpolation equation with three known bands (Dawson and Curran, 1998). It was calculated as follows:

$$\text{REP} = \frac{A\,(\lambda_i + \lambda_{i+1}) + B\,(\lambda_{i-1} + \lambda_{i+1}) + C\,(\lambda_{i-1} + \lambda_i)}{2\,(A+B+C)}$$

Where $A = \frac{D_{\lambda(i-1)}}{(\lambda_{i-1} - \lambda_i)(\lambda_{i-1} - \lambda_{i+1})}$

$B = \frac{D_{\lambda(i)}}{(\lambda_i - \lambda_{i-1})(\lambda_i - \lambda_{i+1})}$

$C = \frac{D_{\lambda(i+1)}}{(\lambda_{i+1} - \lambda_{i-1})(\lambda_{i+1} - \lambda_i)}$

2.3.5 Canopy spectral data collection

Collection of spectral reflectance readings was initiated three weeks after N fertilization until five week after N fertilization. The frequency of data collection was once in a week. Collection of data was done using an Ocean Optics Jaz spectrometer (Ocean Optics, Dunedin, FL).

Jazz spectrometer is a passive sensor and its working principle depends on the passive source of light. The instrument is calibrated against a barium sulfate-coated metal plate (an ideal reflecting surface) and generally reflects greater than 97% of incident light in the range of 340 – 1031 nm wavelength (Ritchie, 2003). It is also necessary to measure the amount of current transmitted from the sensor in the absence of light to calibrate the sensor. In the spectrometer, light comes through fiber optics and gets collimated by a spherical mirror. Then the collimated or parallel rays of light get diffracted by a grating and again focused by a spherical mirror. This light is projected to a linear area charge coupled device (CCD) array. The surface of the CCD contains a photodiode which reacts with light and releases electrons. This release of electrons is positively correlated with the intensity of light or photons that fall on it. Charge coupled device

can hold the charge and systematically pass through the capacitors to the charge amplifier. Then the charge amplifier converts electrons to volts and measures it.

Each of the experimental plots was consisted of three rows: row1, row2, and row3. One 1-m^2 sampling area was marked in each of these rows for each plot with a total of three 1-m^2 areas. The spectral reflectance readings were collected from the marked 1-m^2 area. First, second, and third sensing were done from row1, row2, and row3, respectively. For the sampling of 1-m^2 area, the height of the spectrometer above the crop canopy was calculated as $\tan(\beta/2) = D/H$ formula (β = angular field of view, H= distance of the spectrometer above the matter of interest, D = diameter of area measured). The angular field of view was 28˚. Hence, for measuring 1 m^2 area, the height of spectrometer was 2 m above the crop canopy. But practically, in the field 1-m^2 spectralon plate (coated with barium sulfate) was used to calibrate 1-m^2 area using black plastics. Black plastics were used for creating artificial shade in each side of the board to find out if it was affecting the amount of light reflected from the plate and thus optimum height above the crop canopy was determined. The height above the crop canopy was 1.67 m to cover 1-m^2 area.

2.3.6. Early-season biomass collection and data analysis

Biomass from the entire 1-m^2 area was cut after spectral reflectance reading collection. Fresh weight of the collected early biomass was determined wherein a representative subsample was taken and weighed. The collected subsamples were dried in a greenhouse unit then further dried using an oven at 52ºC for 48 hours. The weight of dried subsamples was then determined. From this information i.e., fresh weight and its corresponding dry weight, the dry biomass yield in kg per hectare was computed.

2.3.7 Statistical analysis

Statistical analysis involved correlation and regression between dry biomass yield and different VIs collected at the same time using software SAS 9.3. Pearson correlation analysis was done for wavelengths 400-850 nm. For 400-600 nm and 780-850 nm, the spectral resolution was 10 nm, while for 600-780 nm, the spectral resolution was 2 nm. Smaller spectral resolution was selected to have more information about the reflectance pattern at wavelength 670 to 780 nm which falls under red-edge.

Regression analysis of early-season dry biomass productions with NDVI, SR (calculated at different wavelengths), and different REP determination procedure was performed.

2.4 Results and Discussion

2.4.1 Wavelength correlation with dry biomass yield

The correlations between dry biomass yield of energy cane and wavelengths for years 2013 and 2014 are reported in Figs. 2.2, 2.3, and 2.4. All three graphs show the relation pattern of canopy spectral reflectance (440-850 nm) with dry biomass production at different sampling times. In 2013, the sampling was done at three times (3, 4, and 5 WAN), whereas in 2014, sampling was done only twice (4 and 5 WAN) due to bad weather. Reflection at visible and NIR wavelengths was found negatively and positively correlated, respectively, with the dry weight of biomass production for both years. This is because green plants absorb light in visible wavebands and reflect light in green and NIR region (Knipling, 1970; Hoffer, 1978; Peñuelas and Filella, 1998; Todd et al., 1998; Mutanga and Skidmore, 2004). Hence, more plant biomass and higher canopy coverage led to higher visible waveband absorption and low reflection and vice-versa. Previous studies also showed that blue, green and red wavebands were negatively correlated with the amount of green biomass production (Colwell, 1974; Drake, 1976; Tucker,

1977; Bartlett and Klemas, 1981; Lorenzen and Jensen, 1998). Reflection at NIR is directly influenced by plant cellular and intercellular structure (Ollinger, 2008). More biomass produces more layers of plant cells and due to internal scattering, a higher degree of reflection of NIR wavebands. The amount of NIR light transmitted though upper layer of leaves is generally reflected back by the lower leaves and retransmitted through the upper layer of leaves which increases the magnitude of reflection at these wavebands (Myers et al., 1966; Knippling, 1970).

Overall, at 4 WAN sampling time, strong correlations were found between reflections at NIR wavebands (700 nm onwards) and biomass dry weight, while 5 WAN showed stronger correlations in visible wavebands among different sampling times both in 2013 and 2014 (Tables 2.2, 2.3, and 2.4). Biomass production and plant coverage were higher at 5 WAN, but the sensitivity of the relationship between canopy spectral reflectance at NIR wavebands and dry weight of biomass was reduced after 4 WAN sampling time. According to Lorenzen and Jensen (1998) the NIR reflection collected from dead leaf and dead plants is negatively correlated with the green biomass. They also found that the magnitude of NIR reflection relation with green biomass was higher than the reflection from total biomass which included dead biomass and standing dead plants. Kanke et al. (2012) mentioned that the reduction of NIR relation at later crop growth stage was due to change in leaf orientation, chlorophyll content reduction along with the crop age in winter wheat. Overall wavelengths of 690 and 740 nm produced the highest negative and positive correlation, respectively, with dry weight of biomass yield due to the reason that chlorophyll absorbs higher light in the red wavelength (690 nm) and reflects more in NIR region (740 nm). Little and Summy (2012) also had similar observation. Therefore, higher biomass had higher quantity of chlorophyll and very low amount of reflection in red waveband (Hansen and Schjoerring, 2003; Nguyen and Lee, 2006). In contrary, NIR reflection depends on

the cellular structure in leaves and more biomass results in more layers of leaves and higher NIR reflection. Later on the relation of VIs, calculated using of 690 nm wavelength, with the dry weight of biomass were analyzed.

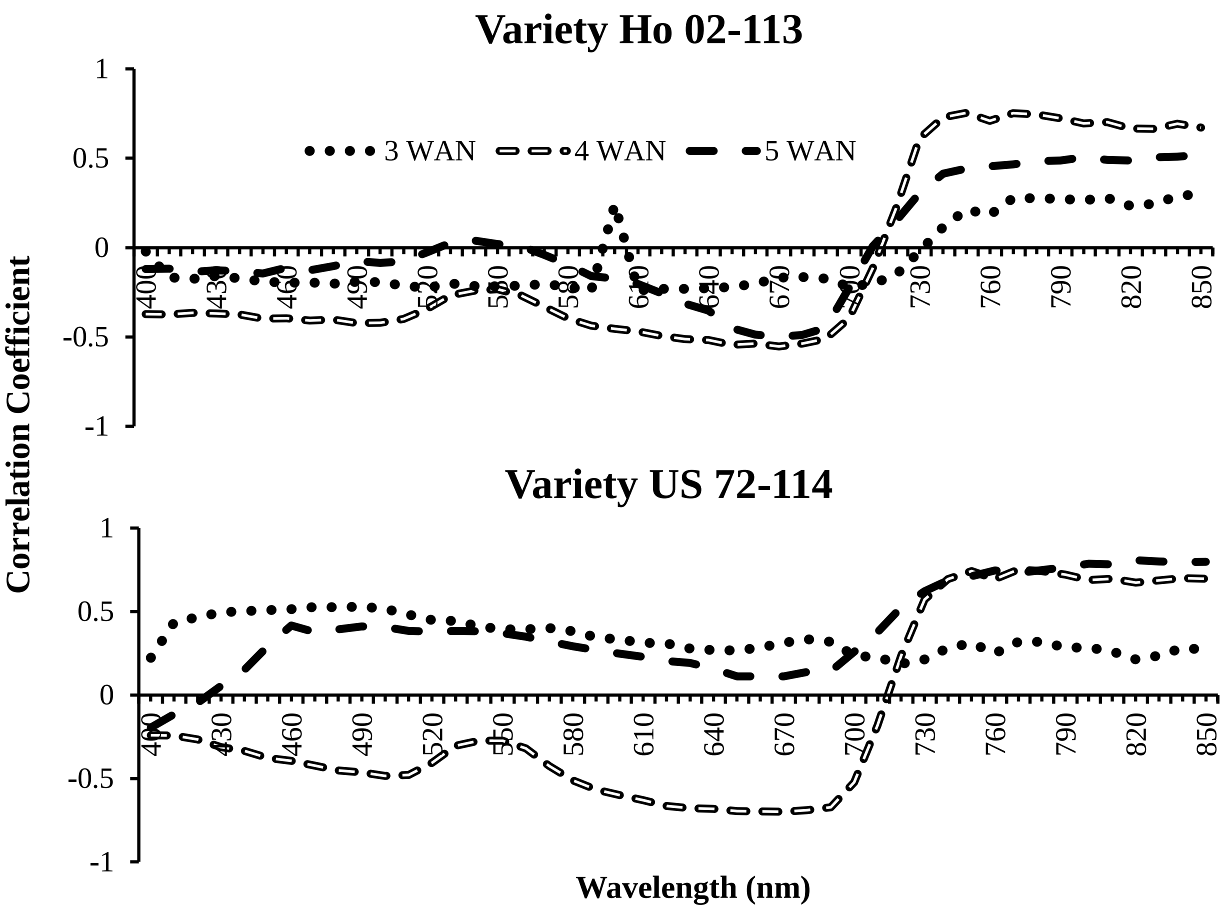

Figure.2.2. Correlation coefficient (*r*) analysis of plant cane biomass yield for two energy cane varieties and wavelength (400-850 nm) in 2013, St. Gabriel, Louisiana.

In 2013, the pattern of canopy spectral reflection relation with biomass yield for the plant cane crop deviated from the normal pattern at 3 and 5 WAN sampling time for variety US 72-114 (Fig. 2.2). Normally, the plant biomass has negative relation with visible wavebands. During 3 and 5 WAN sampling time the relation became positive. The spectral reflectance was affected in variety US 72-114 and the effect was greater in visible waveband than in NIR. This was

probably because the plants were too young at 3 WAN to produce any significant response in canopy spectral reflectance. According to Todd and Hoffer (1998), low canopy coverage produced higher background soil noise and low light reflection by the leaves. Herbicide atrazine at 2.24 kg ha^{-1} was applied as lay-by to control weed in between 4 and 5 WAN which might have produced some stress in those energy cane varieties without producing any visible symptoms.

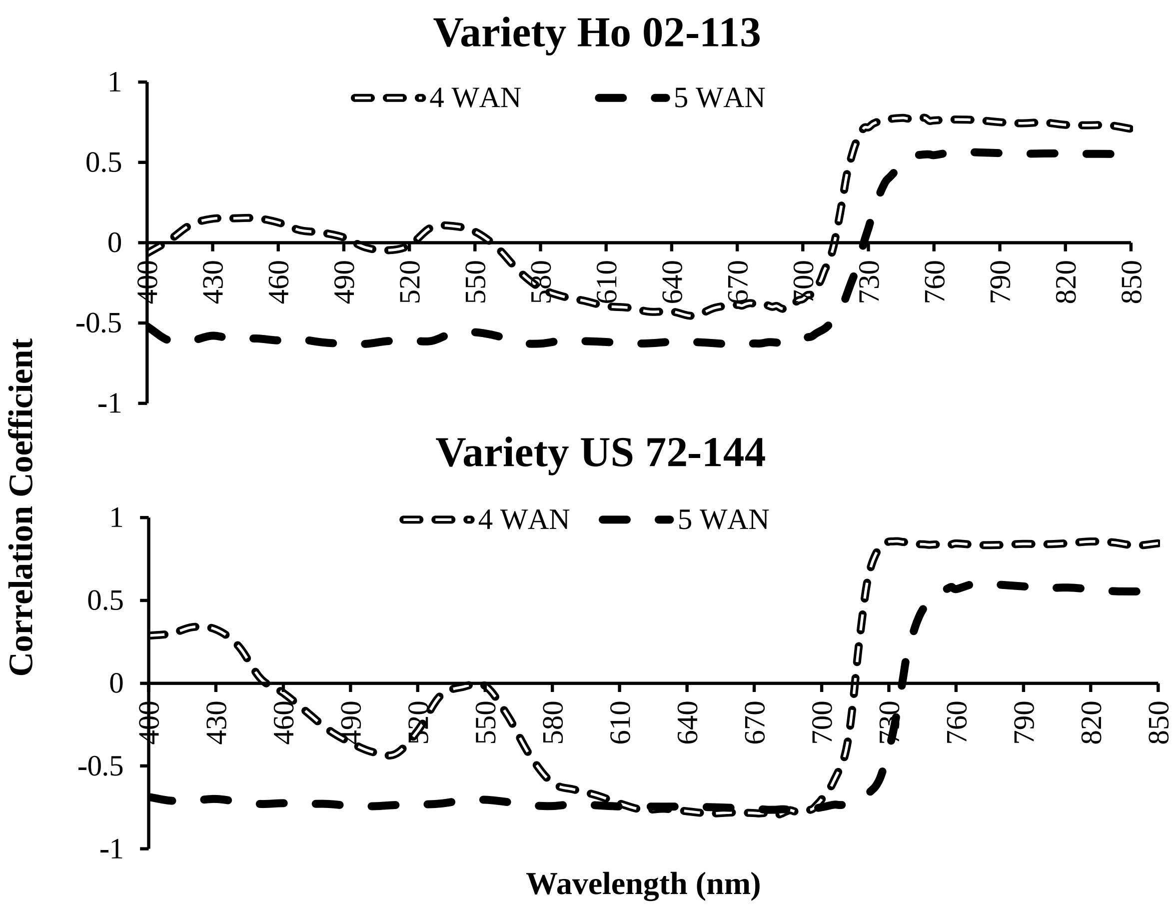

Figure 2.3. Correlation coefficient (r) analysis of first ratoon biomass yield for two energy cane varieties and wavelength (400-850 nm) in 2014, St. Gabriel, Louisiana.

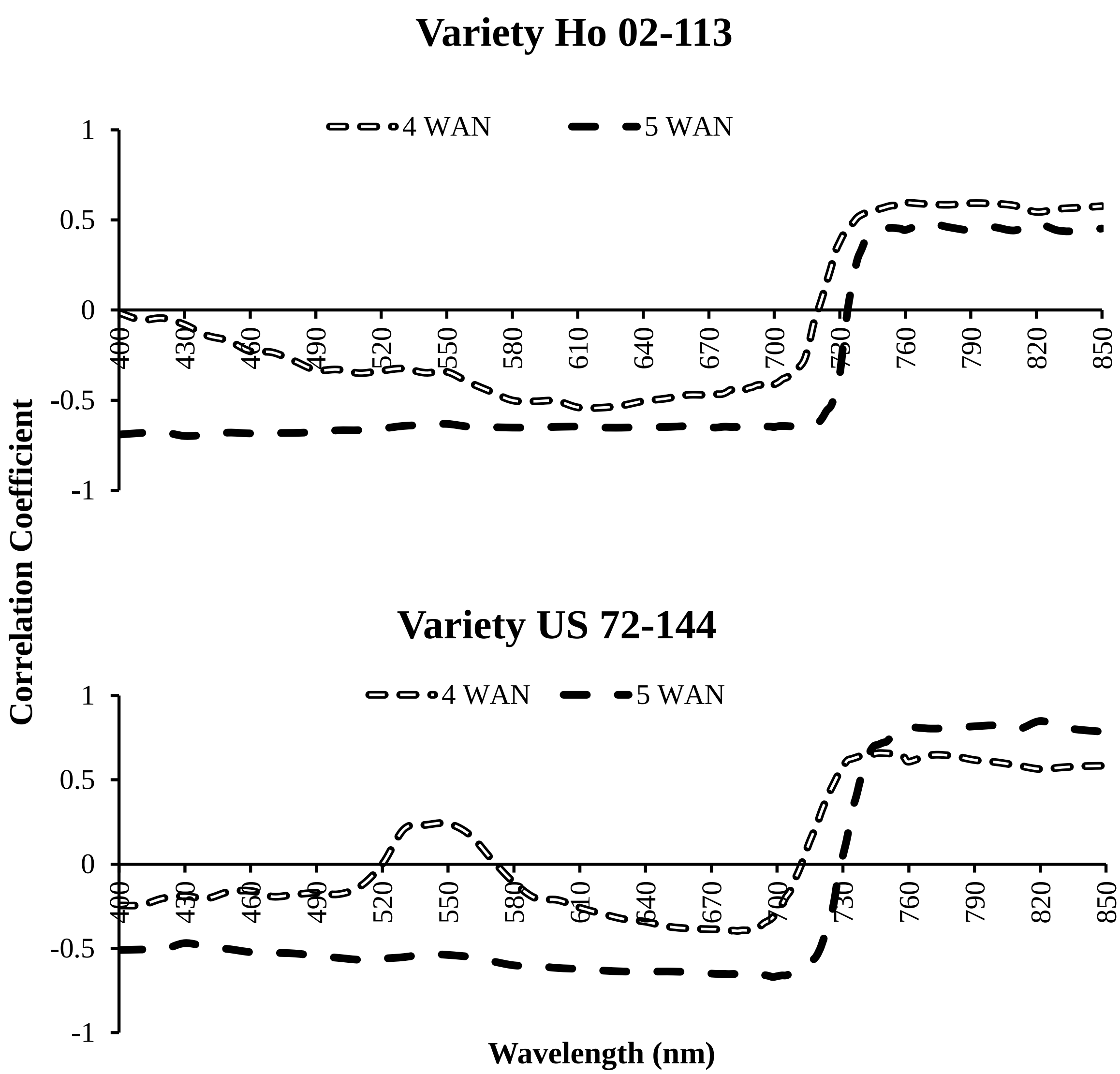

Figure 2.4. Correlation coefficient (*r*) analysis of plant cane biomass yield for two energy cane varieties and wavelength (400-850 nm) in 2014, St. Gabriel, Louisiana.

Carvalho (2009) mentioned that herbicide can induce yield reduction without producing any visual symptom and this reduction of yield can be temporary depending upon crop type and variety. According to Knipling (1970), the reflectance at visible band is generally affected by chlorophyll content which is very much susceptible to physiological stresses. Variety Ho 02-113 at 3 WAN produced normal pattern of reflection where visible and NIR wavebands produced negative relation and positive correlation respectively, although the strength of relationship was

lower than the other two sampling period. Perhaps the reason behind was that variety Ho 02-113 produced 1.5 and 1.3 times higher fresh and dry weight of biomass, respectively, (statistically not significant) than variety US 72-114 at 3 WAN. Higher biomass produced better ground coverage and better relation with spectral reflectance. Overall, there is a small peak in the region 550 nm which falls under green band. Among all visible wavebands, plants absorb all wavebands except green.

2.4.2 Comparison of the strength of relationship between different VI, calculated from reflected light at different wavelengths, and dry biomass yield of energy cane across different sampling times

Wavelengths of red, red-edge, and NIR were selected as 670 (Daughtry et al., 2000; Maness and Stone, 2004), 705 (Sims and Gammon, 2002), and 780 (Maness and Stone, 2004) nm, respectively, for NDVI and SR calculation and are listed in Tables 2.2 to 2.7. For $NDVI_{red-edge}$ and $SR_{red-edge}$ calculations (Tables 2.2 to 2.7), the NIR waveband was 750 nm (Sims and Gammon, 2002). Here coefficient of determination (r^2), probability (*P*- value) and root mean square error (RMSE) values of the regression were considered to determine the relation strength between different vegetation indices and dry biomass yield. The r^2 value defines the strength of relation between two variables and ranges from 0 to 1. Higher r^2 value indicates stronger relationship between the two variables. Probability value determines the level of significance of the relationship between two variables. Root mean square error determines how the models fit with the existing data. It represents the total distance between the predicted value (on regression line) and the observed value (real value). Lower RMSE value indicates closely packed observed value near the regression line and better fit of the model. Although cubic fits better and produced higher r^2 values in most cases, still it cannot represent temporal growth and development of plant (Simões et al., 2009). Hence, it was not accepted for this study.

Overall, dry weight of biomass at 4 WAN produced the strongest relationships with all NDVIs computed using the reflectance readings obtained from different wavelengths (Table 2.2). A similar trend was observed where the reflectance readings from individual wavelength had stronger correlation with biomass at 4 WAN than other sampling times (Fig. 2.2). For both varieties, measured early-season biomass and $NDVI_{red}$ produced a quadratic relation with stronger r^2 (0.40 and 0.59 for Ho 02-113, and US 72-114, respectively), lower RMSE (0.9829 and 0.7675 for Ho 02-113, and US 72-114, respectively), and significant *P*-value ($P<0.05$) than the other sampling times. Maximum possible value of NDVI is 1.0. As the value of NDVI approaches 1, it begins to approach saturation. This is a situation where in red light is almost exclusively absorbed by plant canopy whereas, the NIR is mostly reflected. Hence, at higher biomass, red reflection becomes very close to 0 and NIR becomes higher. Therefore, the value of NDVI approaches 1 and starts to get saturated (Tucker, 1977; Sellers, 1985; Todd et al., 1998; Gao et al., 2000; Thenkabail et al., 2000; Mutanga and Skidmore, 2004). As a result the relation between $NDVI_{red}$ and dry biomass yield changed to quadratic instead of linear. In contrast, Gilbert et al. (1996) obtained a logarithmic relationship between biomass and NDVI where they put X as dry biomass and Y as NDVI. In this experiment, Y is biomass and X is NDVI. For $NDVI_{red\text{-}edge}$, the type of relationship with dry weight of biomass was linear (r^2 values were 0.30 for both Ho 02-113 and US 72-114 varieties). When red-edge (705 nm) was used instead of red (670 nm), the magnitude of absorption was reduced and reflection was increased. Therefore the tendency of NDVI to produce higher values was reduced. As a result, the relationship became linear. At wavelength 690 nm, the type of relation between $NDVI_{690}$ and dry weight of biomass was quadratic with r^2 values 0.41 and 0.46 for Ho 02-113 and US 72-114 varieties, respectively.

Table 2.2. Relationship between early-season dry biomass yield of plant cane and NDVI computed from reflectance reading at different wavelengths in 2013 for site A.

WAN		$NDVI_{red}$			$NDVI_{red\text{-}edge}$			$NDVI_{690}$		
	Ho 02-113									
	Model	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**
3	linear	0.04	2.1222	0.465	0.11	2.0423	0.21	0.06	2.0953	0.349
	logarithmic	0.04	2.1207	0.457	0.11	2.0434	0.212	0.06	2.0934	0.342
	quadratic	0.05	2.1942	0.737	0.11	2.1189	0.468	0.07	2.1659	0.622
	cubic	0.06	2.2655	0.853	0.18	2.1222	0.491	0.14	2.1644	0.588
	exponential	0.04	2.2038	0.780	0.11	2.1219	0.477	0.06	2.1773	0.667
4	linear	0.33	0.9962	0.024	0.30	1.0213	0.035	0.37	0.9700	0.016
	logarithmic	0.35	0.9876	0.021	0.30	1.0202	0.034	0.38	0.9599	0.014
	quadratic	0.40	0.9829	0.046	0.30	1.0624	0.117	0.41	0.9791	0.044
	cubic	0.40	1.0240	0.115	0.31	1.0952	0.221	0.44	0.9975	0.088
	exponential	0.31	1.0560	0.109	0.30	1.0656	0.121	0.35	1.0280	0.078
5	linear	0.33	1.2939	0.020	0.16	1.4526	0.127	0.28	1.3400	0.034
	logarithmic	0.33	1.2975	0.020	0.16	1.4526	0.127	0.28	1.3449	0.035
	quadratic	0.34	1.3342	0.067	0.24	1.4355	0.173	0.30	1.3743	0.098
	cubic	0.35	1.3751	0.143	0.32	1.4098	0.186	0.33	1.4020	0.175
	exponential	0.34	1.3344	0.067	0.17	1.4926	0.287	0.29	1.3796	0.103
	US 72-114									
3	linear	0.03	1.4312	0.538	0.001	1.4505	0.891	0.01	1.4407	0.654
	logarithmic	0.03	1.4309	0.534	0.002	1.4496	0.853	0.02	1.4401	0.644
	quadratic	0.03	1.4848	0.829	0.030	1.4853	0.833	0.02	1.4907	0.873
	cubic	0.14	1.4520	0.590	0.060	1.5186	0.851	0.02	1.5500	0.963
	exponential	0.03	1.4850	0.831	0.001	1.5053	0.991	0.02	1.4948	0.906
4	linear	0.53	0.7925	0.002	0.300	0.9617	0.027	0.43	0.8662	0.006
	logarithmic	0.50	0.8118	0.002	0.290	0.9671	0.030	0.42	0.8768	0.007
	quadratic	0.59	0.7675	0.003	0.300	0.9979	0.096	0.46	0.8820	0.019
	cubic	0.60	0.7901	0.010	0.300	1.0385	0.212	0.49	0.8842	0.037
	exponential	0.57	0.7816	0.004	0.300	0.9995	0.098	0.45	0.8832	0.020
5	linear	0.46	0.9239	0.004	0.300	1.0461	0.028	0.44	0.9360	0.005
	logarithmic	0.47	0.9121	0.003	0.300	1.0461	0.028	0.44	0.9360	0.005
	quadratic	0.51	0.9087	0.010	0.320	1.0703	0.081	0.50	0.9139	0.010
	cubic	0.60	0.8519	0.009	0.390	1.0590	0.107	0.59	0.8626	0.011
	exponential	0.41	0.9957	0.032	0.280	1.1011	0.117	0.40	1.0092	0.038

WAN = weeks after N application, NDVI = normalized difference vegetation index, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Wavelength at 690 nm falls under red wavelength and does not reflect enough light to prevent NDVI from getting saturated. In this case, $NDVI_{red}$ and $NDVI_{690}$ produced stronger

relationship with early-season biomass than $NDVI_{red\text{-}edge}$. Similar finding was observed by Taskos et al. (2013) where $NDVI_{red}$ (630 nm) produced a higher value than $NDVI_{amber}$ (590 nm) and $NDVI_{red\text{-}edge}$ (730 nm) for grapes. Although, 690 nm produced highest negative correlation with dry biomass production, $NDVI_{690}$ failed to produce significantly strong correlation with the dry weight of biomass than $NDVI_{670}$. When the wavelength is greater than 670 nm, the amount of light reflected from the canopy increased which reduces the values of NDVI. As a result, the sensitivity of NDVI or degree of increase or degree of NDVI on the change of biomass was reduced.

Similar trend was observed in case of all SR. Stronger relations between dry biomass yield and SR were observed at 4 WAN as compared to other sampling times. The SR_{red} had quadratic and linear relationship with dry biomass having of r^2 values of 0.39 and 0.59 for varieties Ho 02-113 and US 72-114, respectively (Table 2.3). Variety Ho 02-113 dry biomass yield had a cubic relation with $SR_{red\text{-}edge}$. However, according to Simões et al. (2009) cubic relationship does not represent temporal growth and development of plant. Therefore, linear relationship was the next best choice to explain the pattern of relationship between early-season biomass dry weight and $SR_{red\text{-}edge}$. At 4 and 5 WAN, quadratic model best described the relationship between SR_{690} and dry biomass of Ho 02-113 and US 72-114 with r^2 values 0.35 and 0.55, respectively. Overall, SR_{red} produced better relation with dry weight of biomass as compared to SR calculated with other wavebands i.e., red-edge and 690 nm. Simple ratio computed from the reflectance at the red waveband performed better than reflectance obtained from other wavebands; this was based on its relation with dry weight of biomass.

Table 2.3. Relationship between early-season dry biomass yield of plant cane and SR computed from reflectance reading at different wavelengths in 2013 for site A.

WAN		SR_{red}			$S_{Rred-edge}$			SR_{690}		
					Ho 02-113					
	Model	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**
3	linear	0.03	2.1314	0.518	0.11	2.0481	0.221	0.05	2.1100	0.406
	logarithmic	0.04	2.1255	0.483	0.11	2.0436	0.213	0.06	2.1002	0.366
	quadratic	0.05	2.1870	0.706	0.12	2.1100	0.443	0.09	2.1381	0.526
	cubic	0.05	2.2762	0.880	0.15	2.1499	0.553	0.11	2.2109	0.707
	exponential	0.03	2.2139	0.828	0.10	2.1320	0.507	0.05	2.1942	0.737
4	linear	0.27	1.0396	0.045	0.29	1.0260	0.037	0.31	1.0137	0.031
	logarithmic	0.31	1.0132	0.031	0.30	1.0221	0.035	0.35	0.9861	0.021
	quadratic	0.39	0.9898	0.050	0.30	1.0652	0.121	0.35	1.0225	0.074
	cubic	0.41	1.0172	0.107	0.34	1.0807	0.194	0.52	0.9213	0.039
	exponential	0.25	1.0993	0.176	0.29	1.0723	0.131	0.29	1.0704	0.128
5	linear	0.34	0.2817	0.017	0.18	1.4297	0.097	0.30	1.3209	0.027
	logarithmic	0.34	1.2870	0.018	0.17	1.4451	0.116	0.29	1.3309	0.030
	quadratic	0.35	1.3290	0.064	0.27	1.4087	0.136	0.31	1.3626	0.088
	cubic	0.36	1.3653	0.132	0.29	1.4368	0.227	0.33	1.3998	0.173
	exponential	0.35	1.3286	0.063	0.20	1.4658	0.227	0.31	1.3634	0.089
					US 72-114					
3	linear	0.03	1.4310	0.5350	0.0040	1.4512	0.939	0.01	1.4431	0.692
	logarithmic	0.03	1.4312	0.5370	0.0010	1.4507	0.906	0.01	1.4416	0.668
	quadratic	0.03	1.4843	0.8260	0.0100	1.4960	0.915	0.02	1.4924	0.886
	cubic	0.08	1.5006	0.7790	0.0750	1.5079	0.808	0.02	1.5527	0.971
	exponential	0.03	1.4852	0.8327	0.0004	1.5060	0.997	0.01	1.4973	0.925
4	linear	0.59	0.7393	0.0005	0.3000	0.9653	0.029	0.47	0.8411	0.004
	logarithmic	0.56	0.7653	0.0009	0.3000	0.9618	0.027	0.45	0.8538	0.004
	quadratic	0.60	0.7606	0.0030	0.3000	0.9976	0.095	0.47	0.8669	0.015
	cubic	0.60	0.7916	0.0100	0.3000	1.0373	0.210	0.54	0.8453	0.022
	exponential	0.59	0.7611	0.0030	0.2900	1.0082	0.110	0.47	0.8649	0.015
5	linear	0.38	0.9817	0.0100	0.2800	1.0614	0.035	0.37	0.9932	0.012
	logarithmic	0.43	0.9435	0.0060	0.3000	1.0499	0.029	0.42	0.9549	0.007
	quadratic	0.55	0.8698	0.0050	0.3400	1.0586	0.070	0.55	0.8755	0.006
	cubic	0.58	0.8780	0.0130	0.3800	1.0617	0.111	0.57	0.8819	0.014
	exponential	0.33	1.0630	0.0740	0.2500	1.1215	0.148	0.32	1.0745	0.085

WAN = weeks after N application, SR = simple ratio, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Response of first ratoon biomass yield with different NDVIs was similar in 2014 (Table 2.4). Stronger relationship between early-season dry biomass yield and NDVIs was observed at 4

WAN as compared to 5 WAN. In case of variety Ho 02-113, the relationship of dry biomass production with $NDVI_{red}$, $NDVI_{690}$, and $NDVI_{red\text{-}edge}$ was quadratic with r^2 values of 0.65, 0.67, and 0.71, respectively. Dry biomass yield and $NDVI_{red}$, $NDVI_{690}$, and $NDVI_{red\text{-}edge}$ of US 72-114 had linear relationships with r^2 = 0.71, 0.70, and 0.65, respectively. The r^2, RMSE, and P values were comparable for all the models in variety US 72-114 using $NDVI_{red}$ and $NDVI_{690}$.

Table 2.4. Relationship between early-season dry biomass yield of first ratoon cane and NDVI computed from reflectance reading at different wavelengths in 2014 for site A.

WAN		$NDVI_{red}$			$NDVI_{red\text{-}edge}$			$NDVI_{690}$		
	Ho 02-113									
	Model	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**
4	linear	0.60	0.7239	0.001	0.60	0.7275	0.001	0.62	0.7127	0.0009
	logarithmic	0.59	0.7363	0.001	0.57	0.7588	0.002	0.60	0.7287	0.0010
	quadratic	0.65	0.7126	0.003	0.71	0.6512	0.001	0.67	0.6882	0.0020
	cubic	0.72	0.6695	0.004	0.72	0.6634	0.004	0.72	0.6664	0.0040
	exponential	0.63	0.7323	0.004	0.65	0.7143	0.003	0.65	0.7151	0.0030
5	linear	0.55	0.8685	0.002	0.56	0.8548	0.002	0.36	1.0368	0.0240
	logarithmic	0.55	0.8688	0.002	0.56	0.8548	0.002	0.36	1.0368	0.0240
	quadratic	0.55	0.9055	0.012	0.56	0.8918	0.010	0.37	1.0710	0.0780
	cubic	0.62	0.8708	0.017	0.59	0.9118	0.027	0.38	1.1189	0.1780
	exponential	0.53	0.9213	0.015	0.55	0.9061	0.012	0.36	1.0762	0.0820
	US 72-114									
4	linear	0.71	0.4844	0.0002	0.65	0.5322	0.0005	0.70	0.4936	0.0002
	logarithmic	0.70	0.4918	0.0002	0.63	0.5487	0.0007	0.69	0.5004	0.0002
	quadratic	0.71	0.5019	0.0010	0.66	0.5455	0.0030	0.70	0.5132	0.0010
	cubic	0.71	0.5264	0.0050	0.67	0.5654	0.0090	0.70	0.5383	0.0060
	exponential	0.71	0.5038	0.0010	0.66	0.5471	0.0030	0.70	0.5043	0.0020
5	linear	0.34	0.9383	0.0300	0.42	0.8805	0.0130	0.39	0.9004	0.0170
	logarithmic	0.32	0.9486	0.0340	0.42	0.8805	0.0130	0.39	0.9004	0.0170
	quadratic	0.37	0.9528	0.0770	0.51	0.8389	0.0190	0.47	0.8770	0.0310
	cubic	0.61	0.7890	0.0200	0.69	0.7083	0.0070	0.71	0.6897	0.0050
	exponential	0.36	0.9622	0.0850	0.47	0.8760	0.0300	0.43	0.9047	0.0430

WAN = weeks after N application, NDVI = normalized difference vegetation index, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Variety Ho 02-113 produced 59% higher dry biomass as compared to US 72-114. Variety US 72-114 had greater leaf angle (with the imaginary horizontal line) and longer hypotenuse as

compared to variety Ho 02-113 which indicates comparatively erect canopy structure. Therefore, due to smaller biomass and erect canopy, variety US 72-114 produced better linear relationship between dry early season biomass yield and NDVI as compared to variety Ho 02-113.

Simple ratio showed similar trend as NDVI with early-season dry biomass yield (Table 2.5) and strongest correlation was found at 4 WAN. For variety Ho 02-113, the most suitable model for SR_{red} , SR_{690}, and $SR_{red\text{-}edge}$ to relate dry weight of biomass were linear and quadratic model with r^2 values, 0.63, 0.65, and 0.69, respectively.

Table 2.5. Relationship between early-season dry biomass yield of first ratoon cane and SR computed from reflectance readings at different wavelengths in 2014 for site A.

WAN		**SR_{red}**			**$SR_{red\text{-}edge}$**			**SR_{690}**		
	Ho 02-113									
	Model	***r*²**	**RMSE**	***P*-value**	***r*²**	**RMSE**	***P*-value**	***r*²**	**RMSE**	***P*-value**
4	linear	0.63	0.7014	0.0007	0.65	0.6828	0.0005	0.65	0.6809	0.0005
	logarithmic	0.62	0.7082	0.0008	0.62	0.7120	0.0009	0.64	0.6944	0.0006
	quadratic	0.63	0.7304	0.0040	0.69	0.6701	0.0020	0.65	0.7111	0.0030
	cubic	0.73	0.6559	0.0040	0.74	0.6396	0.0030	0.75	0.6327	0.0020
	exponential	0.61	0.7493	0.0060	0.67	0.6875	0.0020	0.64	0.7216	0.0040
5	linear	0.48	0.9355	0.0060	0.53	0.8832	0.0030	0.36	1.0308	0.0220
	logarithmic	0.53	0.8860	0.0030	0.56	0.8602	0.0020	0.37	1.0300	0.0220
	quadratic	0.57	0.8823	0.0090	0.56	0.8907	0.0100	0.37	1.0729	0.0800
	cubic	0.58	0.9144	0.0280	0.57	0.9290	0.0320	0.38	1.1158	0.1730
	exponential	0.44	1.0140	0.0430	0.50	0.9521	0.0214	0.36	1.0834	0.0890
	US 72-114									
4	linear	0.7	0.4943	0.0002	0.66	0.5244	0.0004	0.68	0.5051	0.0003
	logarithmic	0.71	0.4808	0.0001	0.65	0.5281	0.0005	0.70	0.4917	0.0002
	quadratic	0.71	0.5018	0.001	0.66	0.5476	0.0030	0.70	0.5132	0.0010
	cubic	0.71	0.5262	0.005	0.66	0.5707	0.0100	0.70	0.5381	0.0060
	exponential	0.66	0.5495	0.003	0.65	0.5565	0.0030	0.65	0.5587	0.0030
5	linear	0.39	0.8993	0.017	0.48	0.8303	0.0060	0.47	0.8367	0.0070
	logarithmic	0.36	0.9232	0.024	0.44	0.8652	0.0100	0.42	0.8771	0.0120
	quadratic	0.46	0.8808	0.032	0.58	0.7766	0.0080	0.58	0.7769	0.0080
	cubic	0.58	0.8169	0.028	0.66	0.7382	0.0110	0.68	0.7188	0.0080
	exponential	0.43	0.9123	0.048	0.54	0.8182	0.0140	0.53	0.8253	0.0160

WAN = weeks after N application, SR = simple ratio, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Biomass of variety US 72-114 showed linear relationship with SR_{red}, $SR_{red\text{-}edge}$, and SR_{690} and produced r^2 value of 0.70, 0.66, and 0.68, respectively. Overall, plant cane in 2014 (site B) showed better relation between NDVI at different wavelengths and the dry weight of biomass at 5 WAN then 4 WAN (Table 2.6).

Table 2.6. Relationship between early-season dry biomass yield of plant cane and NDVI computed from reflectance reading at different wavelengths in 2014 for site B.

WAN		$NDVI_{red}$			$NDVI_{red\text{-}edge}$			$NDVI_{690}$		
		Ho 02-113								
	Model	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**	r^2	**RMSE**	***P*-value**
4	linear	0.38	0.5298	0.011	0.34	0.5477	0.018	0.36	0.5403	0.015
	logarithmic	0.38	0.5286	0.011	0.36	0.5388	0.014	0.37	0.5357	0.013
	quadratic	0.39	0.5474	0.042	0.37	0.5522	0.047	0.38	0.5527	0.048
	cubic	0.43	0.5491	0.072	0.42	0.5528	0.078	0.43	0.5476	0.07
	exponential	0.36	0.5608	0.058	0.31	0.5818	0.093	0.33	0.5738	0.078
5	linear	0.48	0.4420	0.003	0.39	0.4765	0.010	0.48	0.4397	0.003
	logarithmic	0.46	0.4477	0.003	0.40	0.4732	0.009	0.47	0.45	0.004
	quadratic	0.48	0.4569	0.014	0.41	0.488	0.033	0.49	0.4542	0.013
	cubic	0.56	0.4385	0.017	0.42	0.5033	0.081	0.56	0.4354	0.016
	exponential	0.46	0.4677	0.019	0.37	0.5024	0.049	0.46	0.4662	0.018
		US 72-114								
4	linear	0.38	0.5104	0.0100	0.32	0.5374	0.023	0.44	0.4889	0.005
	logarithmic	0.35	0.5257	0.0160	0.27	0.5557	0.039	0.40	0.5029	0.008
	quadratic	0.50	0.4757	0.0110	0.42	0.5147	0.029	0.50	0.4758	0.011
	cubic	0.51	0.4911	0.0300	0.42	0.5357	0.080	0.50	0.4951	0.033
	exponential	0.44	0.5038	0.0220	0.36	0.5383	0.052	0.48	0.4890	0.015
5	linear	0.66	0.4486	0.0001	0.70	0.4194	<0.0001	0.68	0.4381	<0.0001
	logarithmic	0.65	0.4575	0.0002	0.68	0.439	<0.0001	0.68	0.4381	<0.0001
	quadratic	0.67	0.4617	0.0008	0.72	0.4201	0.0002	0.68	0.4500	0.0006
	cubic	0.67	0.4746	0.0030	0.73	0.4337	0.0010	0.69	0.4645	0.0023
	exponential	0.67	0.4626	0.0008	0.72	0.4226	0.0003	0.68	0.4508	0.0006

WAN = weeks after N application, NDVI = normalized difference vegetation index, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

The initial crop stand was very low and uneven in this site. As a result, both biomass amount and canopy coverage at 4 WAN were small such that a large portion of the sensor field of view was occupied by soil background. Todd and Hoffer (1998) found that low canopy

coverage produced higher background soil noise and low light reflection by the leaves. In case of variety Ho 02-113, $NDVI_{red}$ and $NDVI_{690}$ performed better than $NDVI_{red\text{-}edge}$. The type of relationship for $NDVI_{red}$, $NDVI_{690}$ and $NDVI_{red\text{-}edge}$ were linear with r^2 value 0.48, 0.48, and 0.39, respectively. For variety US 72-114, dry biomass obtained a better relationship with $NDVI_{red\text{-}edge}$ than $NDVI_{red}$ and $NDVI_{690}$. However, $NDVI_{red}$, $NDVI_{red\text{-}edge}$ and $NDVI_{690}$ had linear model with r^2 value 0.66, 0.70, and 0.68, respectively. The magnitude of biomass production was lowest in 2014 plant cane among all the years and thus the biomass and NDVI produced linear relationship.

Table 2.7. Relationship between early-season dry biomass yield of plant cane and SR computed from reflectance reading at different wavelengths in 2014 for site B.

WAN		**SR_{red}**			**$SR_{red\text{-}edge}$**			**SR_{690}**		
	Ho 02-113									
	Model	**r^2**	**RMSE**	***P*-value**	**r^2**	**RMSE**	***P*-value**	**r^2**	**RMSE**	***P*-value**
4	linear	0.33	0.5509	0.020	0.30	0.5647	0.029	0.30	0.5645	0.029
	logarithmic	0.37	0.5354	0.013	0.33	0.5517	0.020	0.34	0.5472	0.018
	quadratic	0.41	0.5346	0.031	0.40	0.5392	0.035	0.41	0.5349	0.031
	cubic	0.45	0.5402	0.060	0.44	0.5417	0.062	0.46	0.5356	0.055
	exponential	0.29	0.5881	0.107	0.26	0.6008	0.141	0.26	0.6014	0.143
5	linear	0.42	0.4648	0.007	0.36	0.4882	0.014	0.43	0.4616	0.006
	logarithmic	0.46	0.4468	0.004	0.39	0.4787	0.010	0.47	0.4443	0.003
	quadratic	0.52	0.4409	0.009	0.42	0.4846	0.030	0.52	0.4388	0.008
	cubic	0.55	0.4420	0.019	0.42	0.5021	0.078	0.56	0.4384	0.017
	exponential	0.38	0.5000	0.046	0.33	0.5171	0.071	0.38	0.4975	0.043
	US 72-144									
4	linear	0.46	0.4767	0.0040	0.37	0.5185	0.013	0.49	0.4662	0.003
	logarithmic	0.42	0.4969	0.0070	0.33	0.5320	0.020	0.46	0.4796	0.004
	quadratic	0.51	0.4740	0.0100	0.41	0.5183	0.032	0.50	0.4779	0.011
	cubic	0.51	0.4905	0.0300	0.43	0.5318	0.074	0.51	0.4930	0.032
	exponential	0.49	0.4807	0.0120	0.39	0.5258	0.039	0.50	0.4788	0.011
5	linear	0.66	0.4470	0.0001	0.72	0.4092	<0.0001	0.68	0.4350	<0.0001
	logarithmic	0.66	0.4466	0.0001	0.71	0.4162	<0.0001	0.68	0.4359	<0.0001
	quadratic	0.67	0.4600	0.0008	0.72	0.4225	0.0003	0.68	0.4507	0.0006
	cubic	0.68	0.4724	0.0030	0.74	0.4277	0.0009	0.69	0.4620	0.002
	exponential	0.65	0.4748	0.0010	0.72	0.4254	0.0003	0.67	0.4607	0.0008

WAN = weeks after N application, SR = simple ratio, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Similar trend was obtained in SR and dry biomass of plant cane for site B in 2014 (Table 2.7). For variety Ho 02-113, quadratic relationship was obtained between dry biomass yield of energy cane and SR_{red} (r^2=0.52), SR_{690} (r^2=0.52), and $SR_{red\text{-}edge}$ (r^2=0.42). Hence, here also SR_{red} and SR_{690} produced stronger relation than $SR_{red\text{-}edge}$. Biomass of variety US 72-114 had a linear relationship with SR_{red}, $SR_{red\text{-}edge}$ and SR_{690} with r^2 0.66, 0.72, and 0.68, respectively.

2.4.3. Comparison of relation strength between REPs determined using different methods and dry biomass yield of energy cane

Red-edge position determination can successfully be used in remote sensing as a potential tool for yield estimation of high biomass producing crop such as energy cane. Red-edge is the sharp increase from highest absorption wavelength i.e., red to highest reflection wavelength i.e., NIR (Fei et al., 2009). Red-edge position is the wavelength where the highest peak of maximum first derivative is situated (Horler et al., 1983; Boochs et al., 1990; Buschmann and Nagel, 1993; Filella and Penuelas, 1994; Cho et al., 2006). Among the different methods used to determine REP in plant canopy spectral reflectance included, linear interpolation, maximum first derivative, linear extrapolation technique, polynomial fitting technique, and Lagrangian technique were used.

The strength of relationship between REP and dry biomass production of energy cane was evaluated based on r^2, RMSE, and *P*-value of equations. In case of variety Ho 02-113, polynomial fitting calculated an REP which produced the highest correlation with dry biomass yield at 4 WAN with r^2 value of 0.48 (Table 2.8). Red-edge position-computed via linear interpolation also showed highest correlation with dry weight of biomass at 4 WAN and the pattern was linear (r^2=0.37). Similarly, REP computed using Lagrangian method produced quadratic model with r^2 value 0.60 at 4 WAN. At 5 WAN, only REP computed using linear

extrapolation produced the highest correlation with dry biomass and the type of relationship was quadratic (r^2=0.47). Overall, for variety Ho 02-113, REP determined by Lagrangian method produced the strongest correlation with dry biomass compared to the REP determined by the other three methods. For variety US 72-114, the REP (determined by polynomial fitting) and dry biomass at 5 WAN obtained the highest r^2 (0.57) among the sampling times. Similarly, this was also the case for REPs determined by linear interpolation and extrapolation an r^2 value of 0.43 and 0.30, respectively. The highest r^2 (0.39) between dry biomass and REP determined by Lagrangian technique was obtained at 4 WAN. Based on the r^2 value, the REP determined by polynomial regression established better relationship with dry biomass of US 72-114 at 5 WAN. However, the complexities of this procedure may limit its use and application as oppose to a method of REP determination that is simplified by a straight line (linear interpolation).

The REP regardless of determination methods consistently showed the strongest relation with dry weight of biomass production at 4 WAN for both varieties (Table 2.9). For variety Ho 02-113, the REPs determined by polynomial fitting, linear interpolation, Lagrangian, and linear extrapolation had quadratic relationship with dry biomass having r^2 values of 0.33, 0.63, 0.76, and 0.51, respectively while for US 72-114 REPs from linear interpolation, Lagrangian, and linear extrapolation yielded r^2 values of 0.55, 0.54, and 0.64, respectively. Polynomial fitting technique-generated REP also produced strong relationship with dry biomass (r^2= 0.64). Overall, REP determined by Lagrangian technique produced the best relation with dry biomass at 4 WAN for variety Ho 02-113 while it was the linear extrapolation for variety US 72-114.

Table 2.8. Relationship of early-season biomass yield of energy cane plant cane with red-edge position calculated using different methods, site A in 2013.

WAN	Model	Polynomial fitting			Linear interpolation			Lagrangian			Linear extrapolation		
		r^2	RMSE	*P*-value	r^2	RMSE	*P*-value	r^2	RMSE	*P*-value	r^2	RMSE	*P*-value
Ho 02-113													
3	polynomial regression	0.40	0.1080	0.090	0.25	2.0217	0.305	0.36	1.8676	0.133	0.22	2.0675	0.381
	quadratic				0.22	1.9870	0.203	0.36	1.7946	0.0540	0.21	2.0018	0.223
	linear				0.22	1.9152	0.069	0.36	1.7317	0.0140	0.17	1.9665	0.107
	logarithmic				0.22	1.9152	0.069	0.36	1.7315	0.0140	0.18	1.9653	0.106
4	polynomial regression	0.48	0.9198	0.045	0.39	0.9892	0.100	0.60	0.8039	0.0090	0.34	1.0317	0.158
	quadratic	-	-	-	0.38	0.9648	0.047	0.60	0.7736	0.0030	0.18	1.1030	0.267
	linear	-	-	-	0.37	0.9314	0.012	0.56	0.7785	0.0008	0.18	1.0629	0.098
	logarithmic	-	-	-	0.37	0.9314	0.012	0.56	0.7778	0.0008	0.18	1.0629	0.098
5	polynomial regression	0.46	1.2602	0.055	0.36	1.2090	0.167	0.58	1.0148	0.0280	0.50	1.0639	0.046
	quadratic	-	-	-	0.35	1.1634	0.075	0.53	1.0261	0.0160	0.47	1.0505	0.022
	linear	-	-	-	0.22	1.2275	0.080	0.35	1.1538	0.0260	0.42	1.0573	0.009
	logarithmic	-	-	-	0.22	1.2279	0.080	0.35	1.1547	0.0260	0.42	1.0582	0.009
US 72-114													
3	polynomial regression	0.31	1.3053	0.206	0.20	1.4053	0.4351	0.32	1.2951	0.1900	0.40	1.2503	0.115
	quadratic				0.15	1.3846	0.335	0.29	1.2726	0.1120	0.39	1.2116	0.052
	linear				0.15	1.3353	0.133	0.28	1.2309	0.0350	0.22	1.3172	0.079
	logarithmic				0.15	1.3353	0.133	0.28	1.2312	0.0350	0.22	1.3262	0.078
4	polynomial regression	0.37	0.9867	0.123	0.33	0.0212	0.178	0.40	0.9674	0.0990	0.36	0.9951	0.135
	quadratic	-	-	-	0.32	0.9834	0.079	0.39	0.9304	0.0390	0.28	1.0117	0.115
	linear	-	-	-	0.25	0.9925	0.045	0.39	0.8967	0.0090	0.28	0.9766	0.035
	logarithmic	-	-	-	0.26	0.9923	0.045	0.39	0.8968	0.0090	0.28	0.9763	0.035
5	polynomial regression	0.57	0.8821	0.014	0.49	0.9687	0.040	0.32	1.1142	0.1850	0.31	1.1196	0.095
	quadratic	-	-	-	0.43	0.9807	0.026	0.32	1.0706	0.0810	0.30	1.0801	0.091
	linear	-	-	-	0.39	0.9810	0.010	0.31	1.0380	0.0240	0.23	1.0951	0.057
	logarithmic	-	-	-	0.39	0.9807	0.010	0.31	1.0383	0.0250	0.23	1.0964	0.058

WAN = weeks after N application, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Table 2.9. Relationship of early-season biomass yield of energy cane first ratoon with red-edge position calculated using different methods, Site A in 2014.

WAN	Model	Polynomial fitting			Linear interpolation			Lagrangian			Linear extrapolation		
		r^2	RMSE	*P*-value	r^2	RMSE	*P*-value	r^2	RMSE	*P*-value	r^2	RMSE	*P*-value
Ho 02-113													
4	polynomial regression	0.33	1.0352	0.249	0.64	0.7553	0.0140	0.77	0.5995	0.0010	0.51	0.8795	0.057
	quadratic	-	-	-	0.63	0.7291	0.0040	0.76	0.5898	0.0004	0.51	0.8387	0.019
	linear	-	-	-	0.48	0.8303	0.0060	0.70	0.6340	0.0002	0.39	0.8976	0.017
	logarithmic	-	-	-	0.48	0.8310	0.0060	0.70	0.6321	0.0002	0.39	0.8992	0.017
5	polynomial regression	0.30	1.1838	0.290	0.56	0.9408	0.0360	0.35	1.1383	0.2060	0.42	1.0822	0.131
	quadratic	-	-	-	0.56	0.8971	0.0110	0.31	1.1234	0.1320	0.33	1.1020	0.107
	linear	-	-	-	0.56	0.8615	0.0020	0.27	1.1030	0.0560	0.33	1.0560	0.031
	logarithmic	-	-	-	0.56	0.8614	0.0020	0.27	1.1023	0.0550	0.33	1.0560	0.031
US 72-114													
4	polynomial regression	0.64	0.5874	0.013	0.56	0.6553	0.0370	0.60	0.6235	0.0230	0.66	0.5754	0.011
	quadratic	-	-	-	0.55	0.6296	0.0130	0.54	0.6348	0.0140	0.66	0.5488	0.003
	linear	-	-	-	0.45	0.6637	0.0080	0.54	0.6091	0.0030	0.64	0.5394	0.0006
	logarithmic	-	-	-	0.45	0.6640	0.0080	0.54	0.6089	0.0030	0.64	0.5401	0.0006
5	polynomial regression	0.43	0.951	0.115	0.65	0.7494	0.0120	0.50	0.8952	0.0660	0.59	0.8059	0.025
	quadratic	-	-	-	0.53	0.8250	0.0160	0.40	0.9287	0.0580	0.48	0.8647	0.026
	linear	-	-	-	0.41	0.8830	0.0132	0.40	0.8893	0.0150	0.44	0.8600	0.010
	logarithmic	-	-	-	0.41	0.8836	0.0130	0.40	0.8893	0.0150	0.44	0.8600	0.010

WAN = weeks after N application, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

Table 2.10. Relationship of early-season biomass yield of energy cane plant cane with red-edge position calculated using different methods, Site B in 2014.

WAN	Model	Polynomial fitting			Linear interpolation			Lagrangian			Linear extrapolation		
		r^2	RMSE	*P*-value	r^2	RMSE	*P*-value	r^2	RMSE	*P*-value	r^2	RMSE	*P*-value
Ho 02-113													
4	polynomial regression	0.39	0.5654	0.099	0.42	0.5552	0.081	0.70	0.3965	0.002	0.64	0.4360	0.005
	quadratic	-	-	-	0.40	0.5418	0.037	0.57	0.4547	0.004	0.58	0.4504	0.003
	linear	-	-	-	0.40	0.5230	0.009	0.53	0.4614	0.001	0.55	0.4524	0.001
	logarithmic	-	-	-	0.40	0.5230	0.009	0.52	0.4627	0.001	0.55	0.4533	0.001
5	polynomial regression	0.42	0.4833	0.030	0.48	0.4736	0.041	0.50	0.4664	0.035	0.31	0.5463	0.195
	quadratic	-	-	-	0.47	0.4622	0.017	0.41	0.4843	0.030	0.30	0.5305	0.099
	linear	-	-	-	0.35	0.4929	0.016	0.40	0.4727	0.008	0.25	0.5289	0.048
	logarithmic	-	-	-	0.35	0.4928	0.016	0.40	0.4726	0.008	0.25	0.5286	0.048
US 72-114													
4	polynomial regression	0.46	0.5161	0.053	0.48	0.5076	0.044	0.23	0.6163	0.351	0.14	0.6529	0.606
	quadratic	-	-	-	0.46	0.4949	0.018	0.22	0.5959	0.197	0.14	0.6273	0.383
	linear	-	-	-	0.37	0.5172	0.013	0.20	0.5837	0.086	0.13	0.6074	0.172
	logarithmic	-	-	-	0.37	0.5174	0.013	0.19	0.5840	0.087	0.13	0.6076	0.173
5	polynomial regression	0.67	0.4817	0.004	0.68	0.4737	0.003	0.45	0.6248	0.056	0.28	0.7045	0.245
	quadratic	-	-	-	0.67	0.4613	0.0008	0.27	0.6823	0.127	0.24	0.6987	0.173
	linear	-	-	-	0.53	0.5285	0.001	0.26	0.6618	0.043	0.24	0.6737	0.057
	logarithmic	-	-	-	0.53	0.5288	0.001	0.26	0.6619	0.043	0.24	0.6738	0.057

WAN = weeks after N application, r^2=coefficient of determination, RMSE = residual mean square error, *P*-value = probability value.

For site B in 2014, REPs computed using polynomial fitting and linear interpolation showed positive relationship with dry for variety Ho 02-113 at 5 WAN with r^2 value 0.42 (polynomial model) and 0.47 (quadratic model), respectively (Table 2.10). The REPs based on Lagrangian and linear interpolation methods formed quadratic relationships with dry biomass at 4 WAN with r^2 value 0.57 (quadratic model) and 0.58 (quadratic model), respectively. Therefore, REP computed by Lagrangian and linear interpolation methods had better correlation with dry biomass production than the REP generated by the other two methods.

Regardless of method, REP consistently showed better correlation with dry biomass production at 5 WAN than at 4 WAN for US 72-114. Both polynomial fitting and linear interpolation technique-generated REP produced the strongest relationship with dry weight of biomass with r^2 value 0.67. The type of relation between linear interpolation method-generated REP and dry weight of biomass was quadratic.

2.5 Conclusions

Overall, across crop age and varieties, red reflectance-based SR and NDVI at 4 WAN performed best among all VI and produced the highest r^2, lowest RMSE, and significant *P* value. In case of plant cane 2013, dry biomass yield relation with VIs was quadratic for both varieties. First ratoon cane 2014 showed similar trend for both NDVI and SR where the model was quadratic for variety Ho 02-113 and linear for variety US 72-114. Plant cane in 2014 showed better correlation only at 5 WAN because of the thin and uneven crop stand at early crop stage. For variety Ho 02-113, NDVI and SR produced linear and quadratic relationship, respectively, with dry biomass production. Red-edge-based VIs produced linear and the strongest relationship with dry biomass yield of variety US 72-114. In this study, both NDVI and SR consistently

produced quadratic relation with dry biomass across all the cane age. This is expected as NDVI and SR saturate or lose sensitivity with increasing biomass. Methods for determining REP to relate biomass yield performed differently depending on crop variety and age. At 4 WAN for both plant cane (2013 and 2014) and first ratoon (2014), dry biomass of variety Ho 02-113 had the highest correlation with REP determined by Lagarangian technique. For 2013 plant cane, at 4 WAN, dry biomass yield of variety US 72-114 produced the strongest relationship with REP computed by linear interpolation technique. For 2014 plant cane, dry biomass yield of variety US 72-114 showed the best relationship with REP generated by linear interpolation at 5 WAN. In case of first ratoon 2014, dry biomass yield of US 72-114 variety produced best correlation with REP generated by linear extrapolation technique at 4 WAN. Hence among two different varieties, dry biomass of Ho 02-113 consistently produced the best relationship with REP calculated by Lagarangian technique. On the other hand, dry biomass yield of US 72-114 produced highly variable results in correlating with REP determined by different techniques across sampling times and crop ages. In this current experiment, mostly NDVI and SR calculated from reflected light from the red waveband correlated best with early-season biomass than any of those from 690 nm and REP across varieties and site-years hence, still can be useful for high biomass producing crops. However, further research is needed for better interpretation on the relationship between wavelengths and biomass production.

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Chapter 3. Relationship of Early-Season Nitrogen Status of Energy Cane with Total Biomass Yield at Maturity

3.1 Introduction

Nitrogen (N) is one of the major limiting factors for plant growth and production. It is the structural component of living cells, different enzymes, proteins, and chlorophyll, and it has considerable influence on crop biomass production. Nitrogen fertilizer has been used to increase crop production for decades. Its consumption dramatically increased during the 1960s and 1970s mainly due to the decrease in the cost of N (Aldrich, 1980). Urea ammonium nitrate (UAN, 28-32% N) is the most common N fertilizer used for cane in Louisiana. Sugarcane varieties can remove 0.72 kg N t^{-1} of millable stalk production in Louisiana (Golden and Ricaud, 1963; Coale et al., 1993) or 0.76 kg N t^{-1} in Florida (Coale et al., 1993) whereas, it has been reported that energy cane can remove 269 kg N ha^{-1} (Knoll et al., 2012). Increased application of N fertilizers can increase the biomass yield of sugarcane (*Saccharum officinarum*) (Thorburn et al., 2005), switch grass (*Panicum virgatum*) (Kering et al., 2012), and sweet sorghum (*Sorghum bicolor*) (Uchino et al., 2013). Application of N at the rate of 150 kg ha^{-1} also increased the biomass yield and nutritional quality of sugarcane used for forage purpose (Tan, 1995). Biomass yield increases with application of N fertilizer (Day, 1996) as it increases chlorophyll concentration, carbon gain, leaf area, and light-saturated net photosynthesis (Cooke et al., 2005). Nitrogen fertilization decreases root length and root biomass and increases shoot biomass and leaf area (Li et al., 2012). Hence, there is a positive relationship exists between N rate and shoot-root ratio and N content in plant (Agren et al., 1987; Tan et al., 1998; Cambui et al., 2011). Chapin (1980) and Chapin et al. (1987) found similar results from their experiments. In general, the relation between N application and crop yield is quadratic and a decline of crop production is observed after certain dose of N application (Hatfield, 2001). Vos (1997) found that the type of N response

for yield of potato (*Solanum* sp) was not linear. The rate of increase of yield is not same with the rate of increase in N fertilization and it follows the Michaelis-Menten equation. Yield can be increased up to a certain level of N fertilizer application and beyond that limit; the plants either become lodged (Muir et al., 2001) or succulent and susceptible to pest and diseases. Excessive N content in sugarcane results in a prolonged vegetative growth period, delayed ripening, and reduce sucrose content (Legendre et al., 2004; Viator et al., 2013). It also increases reducing sugars (Humbert, 2013) and the non-sugar components of juice leading to poor sucrose recoveries. Not only does excessive N application reduce the yield and quality of crops, it also causes environmental pollution (Good and Beatty, 2011). Nitrogen exists in nature under different oxidational stages (-3 to +5) and can be very difficult to manage as it can be converted into different forms. These forms may be subjected to a variety of loss pathways.

3.1.1 Nitrogen losses from crop fields and their impact

Loss of N from the field does not only cause economic loss but also induce environmental pollution. Nitrate (NO_3^-) leaching from soil causes ground water pollution while gases like nitrous oxide (N_2O) produced from denitrification results in global warming and air pollution (Del Grosso and Parton, 2012; Signor and Cerri, 2013). Nitrogen generally lost from the soil profile through surface runoff, subsurface leaching, ammonia volatilization, ammonia fixation by clay lattice, and denitrification. Nitrate-N is very mobile in the soil and thus can be very easily leached down into the ground water from the soil profile (Randall and David, 2001). It is also reported that repeated application of N fertilizers in agricultural fields can significantly increase the loss of NO_3-N from soil (Gast et al., 1978; Owens, 1990; Jemison and Fox, 1994; Toth and Fox, 1998; Andraski et al., 2000). Nitrate contamination in the ground water can create several human health problems and environmental hazards. The excess NO_3^- can cause wide

spread eutrophication of surface waters, degradation of aquatic ecosystems, increased growth of toxic algal blooms, loss of oxygen, death of fish, and loss of biodiversity (Carpenter et al., 1998). Increased NO_3-N concentration is the main reason for some major human health issues (Keeney and Hatfield, 2001; Follett and Follett, 2001; Pohanish, 2002) like methemoglobinemia or blue baby syndrome in infants and linkages to non-Hodgkin's Lymphoma (Ward et al., 1996). The maximum contamination level (MCL) of NO_3-N, set by United State Environmental Protection Agency (US EPA, 2009) in drinking water is 10 mg L^{-1}. But in many drinking water resources, concentrations of NO_3-N have been found to exceed this limit (Kalita and Kanwar, 1993; Kladivko et al., 1999; Fox et al., 2002).

Nitrogen can also be lost from the field as gaseous ammonia (NH_3) which plays an important role in atmospheric chemistry due to its acid neutralization capacity (Walker et al., 2000). During normal atmospheric conditions, NH_3 reacts with oxides of S and N to form harmful fine particles such as ammonium sulfate [$(NH_4)_2SO_4$] and ammonium nitrate [NH_4NO_3] (Seinfeld and Pandis, 2006; Anderson et al., 2003). These fine particles (Eq 3.1 and 3.2) are mainly responsible for different respiratory problem in human beings when they are deposited in the lungs.

$$2NH_3 + H_2SO_4 = (NH_4)_2SO_4 \quad \text{------------------------------- Eq 3.1}$$

$$NH_3 + HNO_3 = NH_4NO_3 \quad \text{------------------------------ Eq 3.2}$$

Use of slow release N fertilizers (sulfur coated urea, polymer coated urea, triazone, reactive layer coated urea, and shellac-coated urea), nitrification inhibitors (nitrapyrin and dicyandiamide) split and precise N application; can be very helpful in reducing N loss from the soil profile. Crop NUE is highly variable and mainly depends upon N application rate, crop

types, soil types, and weather conditions. It can be defined as the production of grain or crop biomass per unit of N applied in soil (both soil N and N applied as fertilizer). Nitrogen is generally taken up by plants in the form of NH_4^+ and NO_3^- by the roots and through series of physiochemical processes and gets assimilated into plant.

At the end of plant life cycle i.e. after senescence, the assimilated N (organic N) is again converted to inorganic N through mineralization (Eq. 3.3).

$$\text{Inorganic N } (NO_3^-, NH_4^+) \underset{\textbf{Mineralization}}{\overset{\textbf{Immobilization}}{\rightleftarrows}} \text{Organic N } (R\text{-}NH_2) \text{ ---- Eq. 3.3}$$

3.1.2 Different procedures for N uptake measurement

Nitrogen is a structural component of chlorophyll and has a direct correlation with plant chlorophyll content (Van den Berg and Perkins, 2004). Different N measurement procedures can be used to estimate N content in plant, such as tissue analyses (Kjeldahl tissue digestion and Dumas combustion) (Horneck and Miller, 1998), leaf level [leaf transmittance or SPAD meter, leaf color chart (LCC) (Loh et al., 2002; Yang et al., 2003; Netto et al., 2005) and chlorophyll fluorescence (Živčák et al., 2014)], electrical meters (NO_3^- ion selective electrodes and electrical impedance spectroscopy) (Muñoz-Huerta et al., 2013), canopy level reflectance or sensors (ground-based, satellite mounted sensors) (Li et al., 2008; Muñoz-Huerta et al., 2013). In the Kjeldahl tissue digestion method, concentrated sulfuric acid is used. Although this method is reliable, it is very time consuming and hazardous. In the Dumas combustion method, plant tissue is combusted under high temperature producing carbon dioxide and oxides of N. This gaseous N is measured to determine plant tissue N content. This method has also been in use for a long time, but it needs a lot of initial investment. Portable chlorophyll meter has two wavelengths in red and near-infrared (NIR) i.e. 650 and 940 nm, respectively (Pinkard et al., 2006). Plant leaf

absorbs red light and reflects NIR, and the ratio between them is measured and correlated with chlorophyll content in the plant. In chlorophyll fluorescence, the plant re-emits absorbed light. When chlorophyll absorbs light energy, electrons from the outer orbit become excited and moved to a higher energy level. Chlorophyll has blue and red absorption peaks. As blue spectrum has more energy, it shifts the electron to higher energy level than red spectrum. When the excited electron comes to ground state, it emits part of the excess energy in longer wavelength (i.e. dark red color for red absorption band). In healthy plants, this excess energy is trapped in ATP and NADPH (Koning, 1994). Plant sap contains ions which help the current flow through it. Determination of N deficiency through electrical meters is faster than chlorophyll meters. According to Parks et al. (2012) chlorophyll response was observed in sugar beet (*Beta vulgaris* L.) and broccoli (*Brassica oleracea*) depending on variable N rate at 61 and 108 days, respectively, while NO_3^- concentration response in petiole was observed at 47 and 87 days, respectively (Muñoz-Huerta et al., 2013). Different sensors work based on the principle that chlorophyll absorbs red and reflects near infrared (NIR) (Sanderson, 2000). The sensors are mainly of two types: ground based and satellite mounted. The resolution of satellite based sensors is lower than ground based sensors.

Different studies showed that canopy spectral reflectance obtained at certain crop age was correlated with the crop yield at harvest (Rasmussen, 1992; Raun et al., 2001; Teal et al., 2006). Recently, Lofton et al. (2012) established the relationship between canopy reflectance-based vegetation index readings and yield of sugarcane. Few or none of these studies documented the association of early-season biomass production and yield at harvest. Similarly for energy cane, very little is known about the early biomass yield pattern in relation with yield at harvest as affected by different N rates. Keeping all these things in mind, this study was conducted to

evaluate the relationship between early season N-related agronomic variables and yield at harvest (stalk and trash) of energy cane.

3.2 Specific Objectives

1. To evaluate the pattern between early-season biomass production and yield at harvest
2. To evaluate N uptake pattern between early-season biomass production and yield at harvest
3. To monitor different N rates on inorganic soil N content
4. To evaluate the effect of N rates on trash (top and leaves) production of energy cane; and
5. To determine the interactive effects of N rates and energy cane varieties on all measured variables

3.3 Materials and Methods

3.3.1 Experimental site location and characteristics

The study was conducted at two sites at the Louisiana State University AgCenter Sugar Research Station in St. Gabriel, LA. Planting was done in 2012 and 2013 for site A (30º15'47"N 91º05'54"W) and site B (30º16'08"N 91º06'10"W), respectively. The type of the soil was Commerce silt loam (fine-silty, mixed, nonacid, thermic Aeric Fluvaquent). Experimental field was divided in four quadrants and 16 core samples were randomly collected at two different depths (0-15 and 15-30 cm) from each quadrant. Collected soil samples were oven-dried at 52˚C for 36 hours, then ground and analyzed using a CN analyzer for total N and C present in the soil. Mutli-element analyses and pH measurement of the samples were done using Mehlich 3 procedure (Mehlich, 1984) (Table 3.1) and pH meter (1:1 de-ionized water: soil ratio),

respectively. Site A had lower C/N ratio (6:1) as compared to site B (7:1). The soil in site A was slightly acidic (pH=5.5) as compared to the soils in site B (pH = 6.04).

Table 3.1. Background soil characteristics for both sites at LSU AgCenter Sugar Research Station, St. Gabriel, Louisiana.

Properties	**A**	**B**	**Interpretation**
Soil type/texture	Commerce silt loam		-
Soil classification	fine-silty, mixed, nonacid, thermic Aeric Fluvaquent		-
pH	5.5	6.04	Slight acidic
C:N ratio	6:1	7:1	-
Phosphorus, mg kg^{-1}	34	41	High
Potassium, mg kg^{-1}	170	219	High
Magnesium, mg kg^{-1}	458	572	Very high
Sulfur, mg kg^{-1}	10.8	11.7	Medium
Copper, mg kg^{-1}	3.5	4.7	Within critical limit

3.3.2 Plot layout, treatment details, and fertilization of both sites

Planting materials were stalk sections or billets for both of the sites. Billets were prepared by chopping the cane whole stalk with a mechanical combine harvester in such a way that they contained approximately three buds on each piece. The treatments consisted of two energy cane varieties (Ho 02-113 and US 72-114) and four rates of N (0, 56, 112, and 224 kg ha^{-1}) arranged in a split-plot design in a randomized complete block design with four replications where varieties were designated as main plot and N rates were sub plots.

Fertilizer was applied in the month of April in 2013 and 2014 for site A and B. Nitrogen fertilizer i.e. UAN (32% N) was knifed in the soil to minimize volatilization loss (McInnes et al., 1986; Rao and Dao, 1996). The rate of potassium applied was 60 to 80 kg ha^{-1}. Phosphorous fertilizer was not applied as the site was already high in P content.

3.3.3 Biomass sampling and data collection

Sampling was started at three weeks after N application (WAN) with one week interval. A total of three samplings were done from each site every year. Collection of canopy spectral reflectance data was done prior to biomass sampling. Tiller number was counted in 1 m^2 area. Biomass samples were collected from any of the two outer rows of each plot; about one meter away from the end of the row. The fresh weight of whole biomass sample was determined and a known weight of subsample was taken. All biomass subsamples were oven-dried at 52ºC for 48 hours, weighed, ground to pass 1 mm sieve size, and analyzed for total N content by CN analyzer (Model: Vario el cube; Manufacturer: Elementar). Fresh weight of whole biomass and subsample along with the oven-dry weights of subsample biomass were used to compute the early-season biomass yield of energy cane expressed in t ha^{-1}. For computation of yield at harvest, five stalks were harvested manually from each of the three rows and weight was determined. The weight of the stalk and leaves were determined separately to get leaf and stalk ratio. Remaining plot was harvested for millable stalk using, a combine harvester. The plot weight of leaf was computed using leaf stalk ratio and then expressed in t ha^{-1}. Weight of the cut stalk for each plot was determined using a wagon fitted with a load cell. Leaf and shredded stalk samples were collected, oven dried, ground to pass 1 mm sieve size, and analyzed for total N content. Two depths of soil samples (0-15 and 15-30 cm) were collected after harvest. Soil samples were dried at 52ºC and analyzed for inorganic N content (NO_3^- and NH_4^+) using 1.0 M KCl extraction procedure (Keeney and Nelson, 1982) followed by automated flow injection analysis (Lachat QuickChem 8500 Series 2) (Riley et al., 2001).

Analysis of variance (ANOVA) was performed using PROC MIXED in SAS 9.3 (SAS Institute, 2012) to determine the effect of variety, N rate, and their interaction on all measured

variables. Mean separation was done by Tukey–Kramer post-hoc test. Homogeneity of variance was tested by Bertlett method before pooling the data (Bartlett, 1937).

3.4 Results and Discussion

3.4.1 Biomass yield response pattern between early growth stage and harvest

Variance was homogeneous for N rates and varieties at early stage on tiller production, biomass yield and N uptake (Table 3.2). There was no significant interaction effect between variety and N rate observed for all measured plant parameters at the early stage of growth. Therefore, the response of varieties across different N rates was constant. There was no significant difference in tiller production across N rate and variety. Test for HOV using Bartlett method was also done (Table 3.3). Variance was homogeneous across crop age and sites. At harvesting, there was no interaction effect between varieties and N rate for measured parameters in 2013 plant cane and 2014 first ratoon cane. In 2014 plant cane, there is interaction effect between N rate and varieties. Hence, for 2014 plant cane only, the N effect on biomass yield and N uptake were presented separately by variety.

Table 3.2. Summary of analysis of variance (ANOVA) showing level of significance for all measured plant parameters at early stage of growth of energy cane.

WAN	Treatments	Tiller number sqm^{-1}	Biomass yield t ha^{-1}	N uptake kg ha^{-1}
		P-value	*P*-value	*P*-value
2013 Plant cane				
3	Variety	0.368	0.179	0.176
	N rate	0.446	0.348	0.557
	N rate*variety	0.282	0.533	0.469
	HOV (Bartlett)		0.133	0.096
4	Variety	0.615	0.431	0.141
	N rate	0.277	0.589	0.055
	N rate*variety	0.564	0.987	0.643
	HOV (Bartlett)		0.935	0.214
5	Variety	0.672	0.129	0.106
	N rate	0.944	0.354	0.065
	N rate*variety	0.622	0.834	0.963
	HOV (Bartlett)		0.373	0.182
2014 First ratoon				
4	Variety	0.194	0.005	0.004
	N rate	0.326	<0.0001	<0.0001
	N rate*variety	0.817	0.681	0.841
	HOV (Bartlett)		0.382	0.684
5	Variety	0.383	0.003	0.016
	N rate	0.103	0.002	<0.0001
	N rate*variety	0.864	0.69	0.0651
	HOV (Bartlett)		0.719	0.887
2014 Plant cane				
4	Variety	0.107	0.063	0.063
	N rate	0.0002	0.083	0.083
	N rate*variety	0.568	0.707	0.707
	HOV (Bartlett)		0.898	0.378
5	Variety	0.663	0.980	0.980
	N rate	0.011	0.061	0.061
	N rate*variety	0.909	0.800	0.800
	HOV (Bartlett)		0.898	0.378

WAN = weeks after N application. N rate = nitrogen rate, HOV = homogeneity of variance, Bartlett = a test for homogeneity of variance, *P*-value = probability value.

Table 3.3. Summary of analysis of variance (ANOVA) showing level of significance for measured plant parameters at harvest in energy cane.

Treatments	Biomass yield t ha^{-1}	N uptake kg ha^{-1}	Leaf yield t ha^{-1}
	P-value	*P*-value	*P*-value
2013 plant cane			
Variety	<0.0001	0.004	<0.0001
N rate	0.014	<0.0001	0.205
N rate*variety	0.078	0.248	0.851
HOV (Bartlett)	0.629	0.063	0.133
2014 first ratoon cane			
Variety	0.03	0.324	0.176
N rate	0.007	<0.0001	0.296
N rate*variety	0.159	0.21	0.486
HOV (Bartlett)	0.485	0.299	0.786
2014 plant cane			
Variety	0.051	0.537	0.749
N rate	<0.0001	<0.0001	0.006
N rate*variety	0.017	0.012	0.081
HOV (Bartlett)	0.864	0.616	0.237

N rate = nitrogen rate, HOV = homogeneity of variance, Bartlett = a test for homogeneity of variance, *P*-value = probability value.

In the following graphs (Figs. 3.1, 3.2, and 3.3), the response pattern to N rates of early-season biomass yield and yield at harvest is demonstrated. Bars with the same letters, were not significantly different (*P*=0.05).

There was no significant early-season biomass yield response to N across sampling times in 2013 plant cane (Fig. 3.1). This might be due to the application of herbicide atrazine done between 4 and 5 WAN to control weeds. The application might have resulted in burning of the edges of top most leaves of energy cane. However at harvest, the effect of N rates fertilizer on cane tonnage yield was observed which indicates that the cane had recovered from the herbicidal effect at the later growth stage. Similar thing was mentioned by Carvalho (2009) where herbicide can induce yield reduction without producing any visual symptom and this reduction of yield can be temporary depending upon crop type and variety.

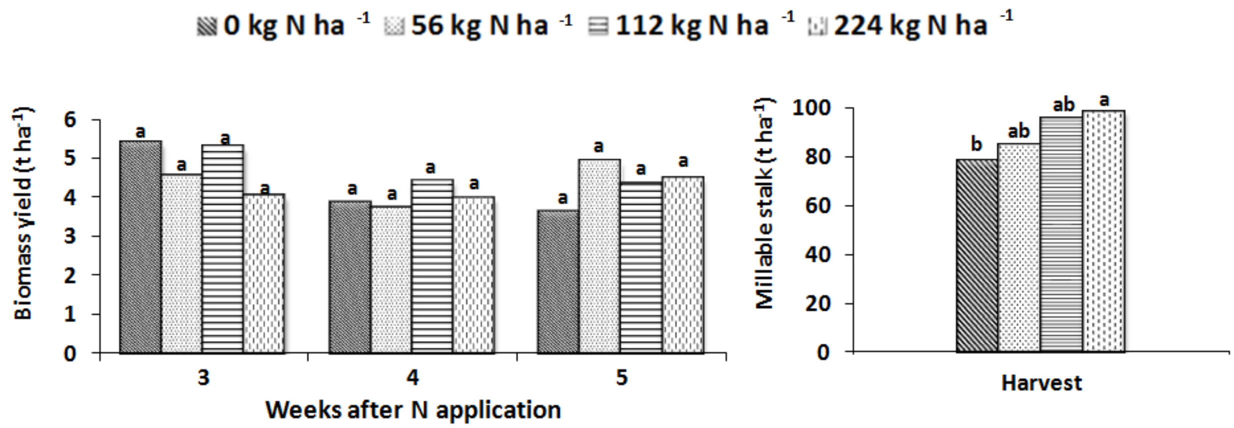

Figure 3.1. Pattern of yield response to nitrogen application at early stage of growth and harvest of two energy cane varieties in 2013 plant cane.

. Although the early-season biomass yield response to N was not significant, there were some interesting patterns found in the data. At harvest, the application of 224 kg N ha^{-1} produced significantly higher yield i.e., 1.25 times higher yield than control (P<0.05). However, none of the early sampling time showed similar pattern. In fact at 3 WAN the control produced the highest yield. Four WAN sampling time showed some similarities in terms of N response pattern with that of harvest. The lowest yield at 4 WAN was obtained with the application of 56 kg N ha^{-1} rate while at harvest, control plot produced the lowest yield. Nitrogen rate at 112 kg ha^{-1} produced highest biomass yield (1.11 times higher) followed by 224 kg N ha^{-1} at 4 WAN. Similarly, 112 and 224 kg ha^{-1} N rate produced almost similar stalk yield i.e. 95 and 98 t ha^{-1}, respectively at harvest. At 5 WAN, the highest biomass yield (4.9 t ha^{-1}) was produced by plots applied with 56 kg N ha^{-1}. In contrast, at harvest it produced the second lowest yield. Control had the lowest yield recorded both at 5 WAN and harvest.

In 2013, the data were collected at three sampling times (3, 4, and 5 WAN) while in 2014, there were only two sampling times (4 and 5 WAN). Due to the adverse climatic condition, no data was obtained at 3 WAN in 2014. In 2014 first ratoon, biomass response to N

application at 4 and 5 WAN showed similar pattern as the millable stalk recorded at harvest (Fig. 3.2). In 2014, weeds were controlled mechanically (pulling, cutting, and burying in the soil) avoiding the potential damage of chemicals as what observed in the previous year. In all sampling times, 224 kg N ha^{-1} produced significantly higher biomass yield than control plot. There was a stepwise increase in biomass yield at early sampling time with increasing N rates.

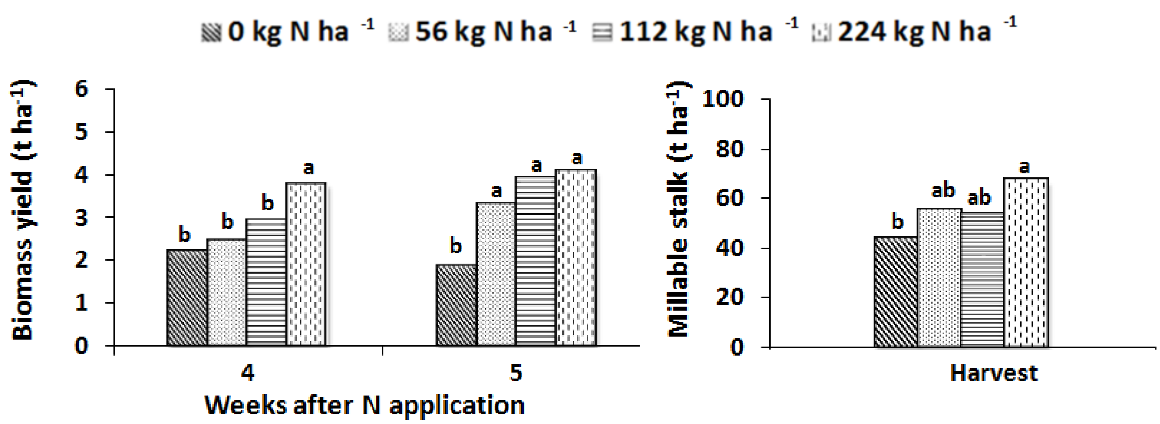

Figure 3.2. Pattern of yield response to nitrogen application at early stage of growth and harvest of two energy cane varieties, in 2014 first ratoon cane.

At 4 WAN sampling time, 224 kg N ha^{-1} produced significantly higher yield at 3.78 t ha^{-1} than the rest of plots which received lower N rates. At 5 WAN, all plots treated with N produced significantly higher biomass yield than control plots. There was no significant difference between 56 and 112 kg N ha^{-1} across sampling times.

Early-season biomass for both varieties did not respond to N (Fig 3.3). The lack of response may be due to relatively high amount of available N in the soil (52 kg N ha^{-1}) prior to N application. Both of the early-season sampling showed that biomass yield tended to increase with increasing N rate, the same trend at harvest except the increase due to N at this sampling time were significant (P<0.05). For variety Ho 02-113, yields of control plots early in the season were numerically lower than the N-fertilized plots, which was similar at harvest where control plot

produced significantly lower cane tonnage than 112 and 224 kg N ha^{-1} treated plots. Similar observations were obtained for variety US 72-114 wherein biomass yield of N-fertilized plots were numerically higher than the control plots. Increases due to N application became significant only at harvest (P<0.05).

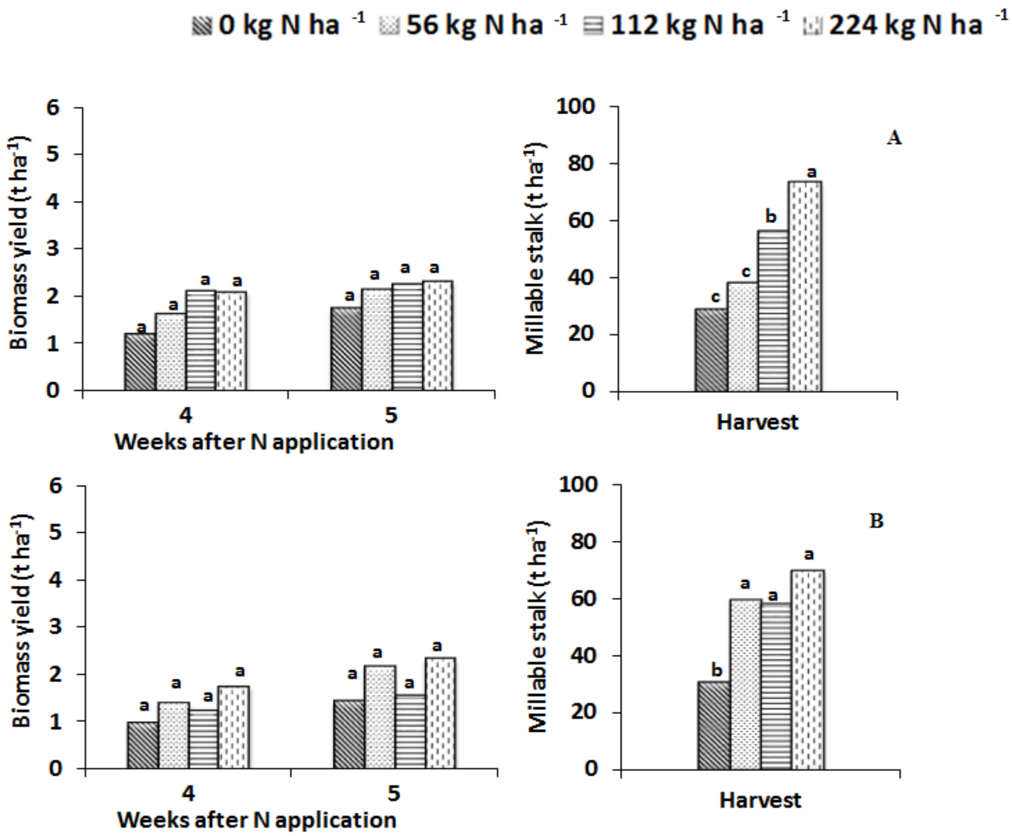

Figure 3.3. Pattern of yield response to nitrogen application at early stage of growth and harvest of two energy cane varieties Ho 02-113 (A) and US 72-114 (B), in 2014 plant cane.

3.4.2 Nitrogen uptake response pattern between early growth stage and harvest

Nitrogen rate did not affect N uptake across sampling times in 2013 plant cane (Fig.3.4). This was due to the effect of atrazine herbicide applied between 4 and 5 WAN at 2.24 kg ha^{-1} which produced canopy burning and subsequently induced some physical stress and thus suppressed the effect of N rate on N uptake. However, the crop recovered from the herbicide effect and showed stepwise increment of N uptake with increasing N rate at harvesting. The

application of 224 kg N ha^{-1} resulted in significant increase in stalk N uptake by as much as 67 kg ha^{-1} as compared to other N-fertilized plots ($P<0.05$). At 3 WAN, the control plot produced 11 and 5 % higher N uptake than the plots received 56 and 224 kg N ha^{-1}, respectively. Nitrogen rate at 112 kg ha^{-1} produced maximum N uptake of 53 kg ha^{-1}. Four WAN showed similar pattern of N uptake with harvest at N rates. Both early-season sampling time and harvest produced stepwise increase in N uptake along with increase in N rate.

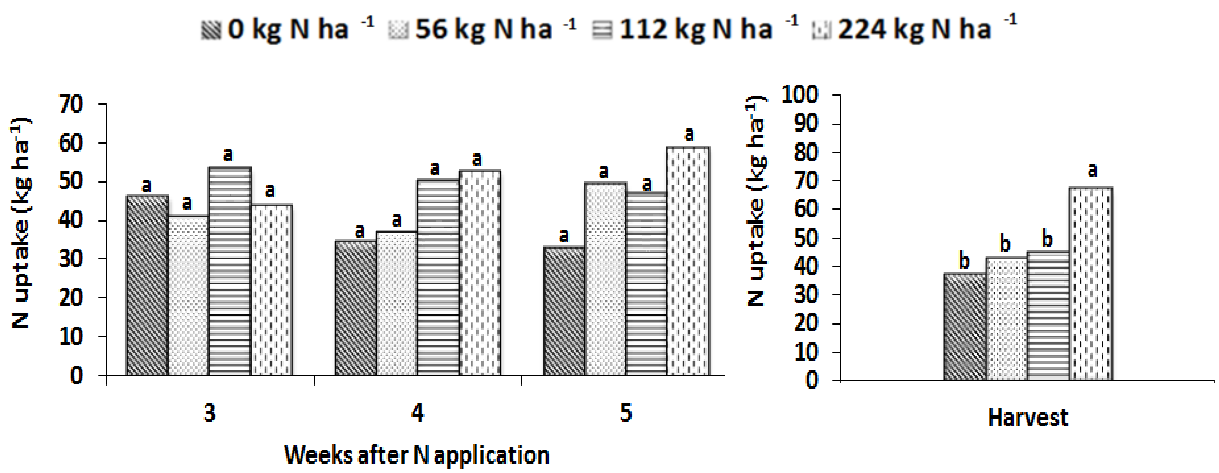

Figure 3.4. Response pattern of nitrogen uptake to nitrogen application at early stage of growth and harvest of two energy cane varieties in 2013 plant cane.

There was 1.5 time increase in energy cane N uptake brought about the application of 224 kg N ha^{-1} using control as reference. Similar trend of N uptake was observed at 5 WAN sampling time and harvest where N uptake increased with N rate except at 112 kg N ha^{-1} which produced lower value than 56 kg N ha^{-1}. Overall, plots applied with 224 kg N ha^{-1} had the highest N uptake compared with the rest of the N rates.

For the 2014 first ratoon, cane N uptake at 4 and 5 WAN was significantly influenced by N rate ($P<0.05$) at different N rates (Fig. 3.5). There was a consistent increase in N uptake with increasing N rate for both early-season sampling times.

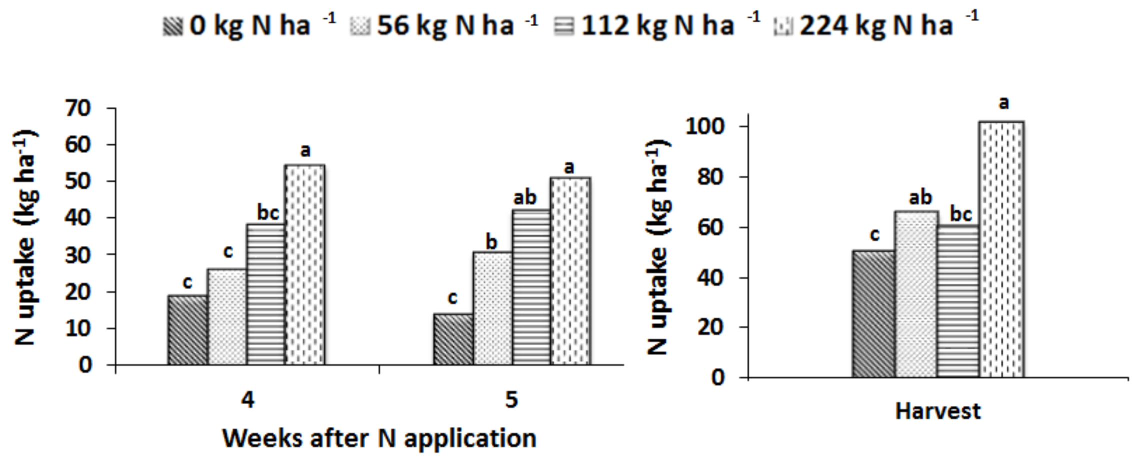

Figure 3.5. Response pattern of nitrogen uptake to nitrogen application at early stage of growth and harvest of two energy cane varieties in 2014 first ratoon cane.

Across sampling time including harvest, plots fertilized with 224 kg N ha^{-1} produced higher N uptake than the control plots (P<0.05). At harvest, there was a gradual increase in N uptake except at 112 kg N ha^{-1} treated plots which produced 8 % lower N uptake than the plots applied with 56 kg N ha^{-1}. Results showed that the pattern of N response at harvest were similar with the early sampling stages (4 and 5 WAN) of energy cane production.

For 2014 plant cane, there was no significant N rates effect observed on early-season N uptake pattern in both varieties (Fig.3.6). However, it is notable that the pattern of N uptake was increasing with N rate. The initial available N in the soil was high (52 kg N ha^{-1}) thus, the increase in N uptake brought about by applying additional N was minimal. Over time soil N was utilized by cane but not enough throughout the cropping year. At this point, the added N to N-treated plots became beneficial which in turn significantly raised the N uptake of millable stalks at harvest.

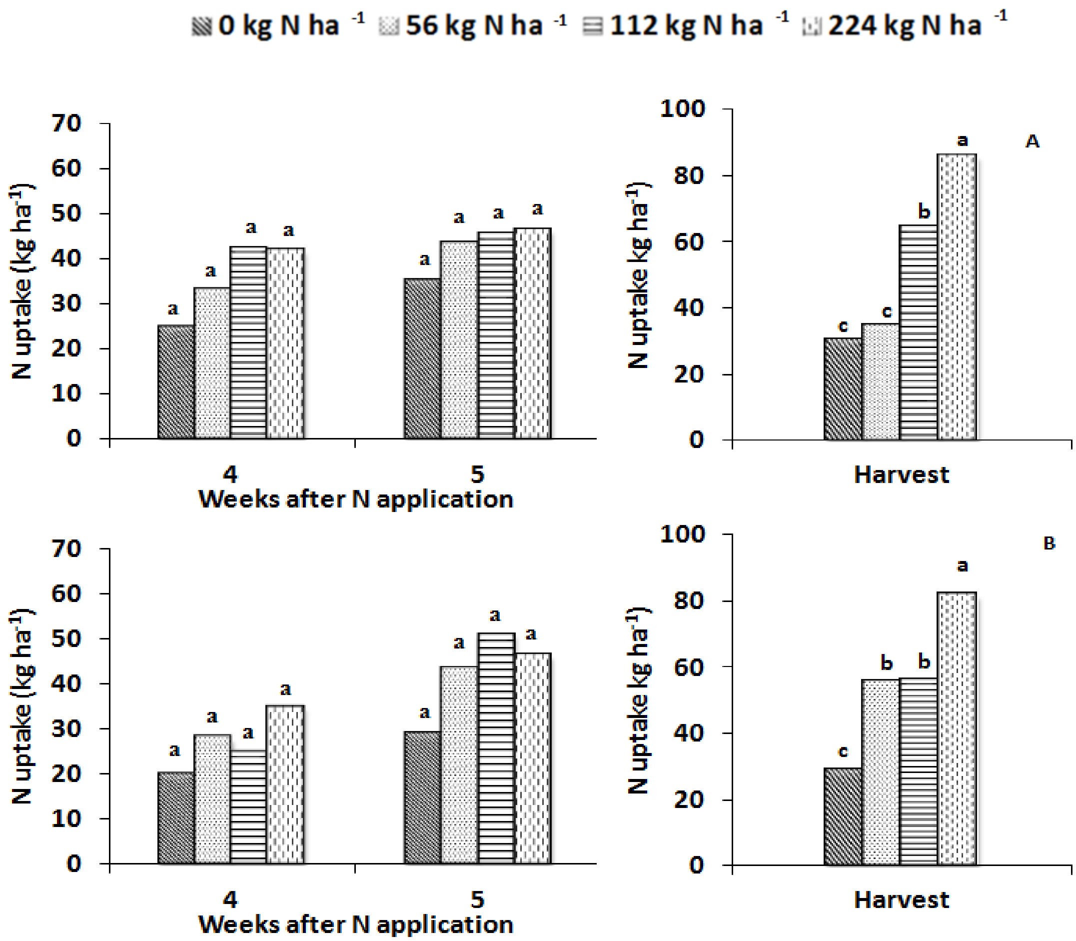

Figure 3.6. Response pattern of nitrogen uptake to nitrogen application at early stage of growth and harvest of two energy cane varieties Ho 02-113 (A) and US 72-114 (B) in 2014 plant cane.

3.4.3 Dry leaf yield affected by N rate

For both 2013 plant cane and 2014 first ratoon crop, the effect of N rate on dry leaf yield was not significant (Table 3.2). Numerically, plots fertilized with 112 kg N ha^{-1} produced the highest amount of dry leaf while and control plots produced the lowest. In 2014 first ratoon, 56 kg N ha^{-1} produced the numerically highest amount of leaf (5.66 t ha^{-1}) dry weight, while the lowest leaf yield was produced by plots applied with the highest N rate i.e. 224 kg N ha^{-1}.

Table 3.4. Analysis of variance (ANOVA) on leaf yield of two cane varieties treated with different N rate.

N rate (kg ha^{-1})	[1]Dry yield of leaf (t ha^{-1})		
	2013 plant cane	**2014 1st ratoon**	**2014 plant cane**
0	10.329^{a}	2.9284^{a}	5.0422^{b}
56	10.8397^{a}	6.1038^{a}	7.2772^{ab}
112	12.8556^{a}	5.6661^{a}	7.6019^{ab}
224	12.1247^{a}	2.6291^{a}	9.8664^{a}

[1]The means within a column sharing the same letter are not significantly different (*P*=0.05).

Normally, energy cane harvesting includes both leaves and stalks (Bassam, 2010). According to Franco et al. (2011) removal of 66 % dried leaves from field to processing unit would not harm soil fertility level as the top leaves contain more nutrient than dried leaves and helps nutrient recycling in soil. Cane leaves contain high amount of cellulose and hemicellulose that can be converted into bioethanol and due to trashing or burning leaves, we are losing tons of potential biomass for ethanol production. According to Khadoo- Jeetah (2013) 1 ton cane leaves and top can produce 259 liters of bioethanol.

3.4.4 Effect of different N rates on total inorganic N content in soil after harvest

Overall, surface soil layer i.e. 0-15 cm had higher total inorganic N than the deeper (15-30 cm) soil layer except in 2014 plant cane (Fig. 3.7). Soil samples were taken from the same plots for 2013 plant cane and 2014 first ratoon. During the first cropping year (2013 plant cane), there was no significant difference in inorganic N present in soil at both soil depth. However, for 2014 first ratoon (second cropping year), soil samples collected from 224 kg N ha^{-1} treated plots had significantly higher inorganic N content at 0-15 cm soil layer compared with the rest of the N rates. Control plots had the lowest inorganic N content which is 1.9 times lower than 224 kg N ha^{-1} treated plots. This result was quite expected as higher N rate in the form of UAN (readily available fertilizer) leads to higher available inorganic N content in soil.

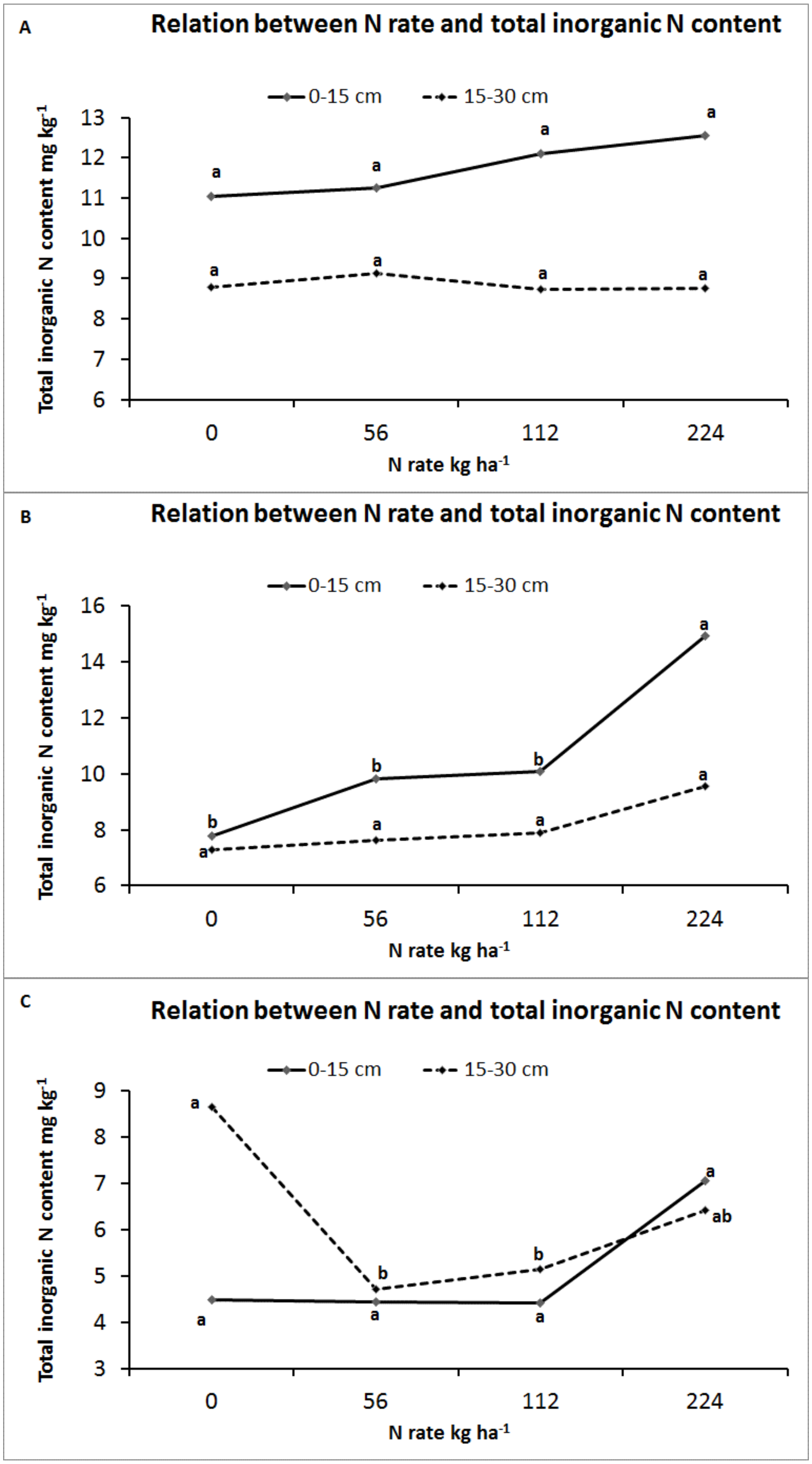

Figure 3.7. Effect of different N rates on total inorganic N content in soil after harvest for different crop season i.e. 2013 plant cane (A), 2014 first ratoon (B), and 2014 plant cane (C).

Lasa et al. (2011) also found similar trend of result regarding mineralized N content in soil using sprinkler and furrow irrigation system. Similar trends were observed for deeper layer of soil but the N fertilization effect was not significant on soil N content. There was 13 % increase in total inorganic N content than using control plots as reference.

For 2014 plant cane, similar trend was observed with little (58 %) increase of inorganic N content at 224 kg N ha^{-1} treated plot than control in surface layer of soil. However, there is a big increase in inorganic N content of the control plots at deeper soil layer than the surface layer. Similarly, 224 kg N ha^{-1} also produced higher yield than the other N application rates. In this site the deeper layer contained higher inorganic N content than surface layer. This was partly attributed to the heavier texture of the soil in this site than the other site which can favor higher runoff than the infiltration rate. The amount of rainfall received in 2014 was above the average rainfall of the state which may have led to losses of applied N from surface soil.

3.5 Conclusions

Overall, N application rates significantly affect the N uptake and biomass production of energy cane over the years during harvesting. However, the highest dose of N did not consistently and necessarily produce the highest yield which indicates that an appropriate estimation of N application is necessary to improve NUE and minimize N loss from the soil profile. Results also showed that background N concentration in the soil can affect the relationship between N application rate and biomass production. If the background N concentration is high in the soil, there may be no effect of applied N on biomass production. The same phenomenon was observed at harvesting when background soil N concentration was depleted and 224 kg N ha^{-1} application rate performed significantly better than the control plots for all site years. Hence, it clearly imposes that background soil testing is a potential tool for

judicious N application in the soil and eventually for better decision making policies in crop production. This experiment also showed that higher N rate produced higher total inorganic N content in surface soil for all site years. Leaves can be a valuable source for bioethanol production due to the presence of cellulose, hemicelluloses, and lignin in it. Increasing N application rates had no significant effect on leaf production, except for plant cane in 2014 where the application of 224 kg N ha^{-1} resulted in significantly higher leaf yield than unfertilized energy cane.

The outcome of this study showed that there were similarities in the pattern of early-season N response to N rates and pattern of yield response to N at harvest. The N response pattern at 4 and 5 WAN sampling stages showed similarity to the N response pattern at harvest. These early-season samplings were done at 4 and 5 WAN, and were within the period where N fertilizer is applied to cane in Louisiana. Based on the results of this study, assessing N responsiveness of cane early in the season using these variables can be a viable approach to refine current N recommendation in Louisiana sugarcane production systems. Further, this relation between early-season and harvest stage can also explain the applicability of early season canopy spectral reflectance collections to estimate the yield at harvest.

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Chapter 4. Using Vegetation Index Readings and Its Coefficient of Variation to Estimate Cane Stand Population as Affected by Planting Method

4.1 Introduction

Coefficient of variation (CV) is a statistical measurement to determine dispersion or variability in the data and is calculated using the following formula:

$$\mathrm{CV} = \frac{\text{Standard deviation}}{\text{Mean}} \text{ x } 100$$ (Pearson, 1895; Bowman, 2001)

Coefficient of variation is dimensionless and expressed as percentage and can be used to compare variability among different units. Normalized difference vegetation index (NDVI) is one of the most widely used vegetative indices for predicting crop stand. Rouse et al. (1973) was the first one who used NDVI after the launch of the satellite ERTS-1. It is a ratio of vegetation index which uses subtraction of red and near infrared (NIR) reflection from crop canopy as numerator and addition of these two as denominator. As it is normalized, it produces very stable values across different canopy structure and degree of greenness (Ramachandran et al., 2011).

Coefficient of variation among NDVI readings can be used to determine species richness (type of different species of plants present in certain area), make decision for the validity of treatments, and to access plant population stand variability. It has been reported that the mean NDVI readings have positive correlation with species richness (Gillespie, 2005; Levine et al., 2007; Seiferling et al., 2012). This is because NDVI at higher resolution of satellite image (1 km) is associated with evapotranspiration and net primary productivity of plants (Currie and Paquin, 1987; Levine et al., 2007). Variance among NDVI readings are negatively correlated with species richness (Gillespie, 2005; Levine et al., 2007; Seiferling et al., 2012).

Commonly, validation of treatments is done using least square difference. Some scientists also used CV for validation of different treatment assuming mean and CV are positively correlated (Allen et al., 1978; Gotoh and Osanai, 1959; Bowman, 2001). If size of the mean increases CV also increases and vice-versa. In contrast, according to Bowman and Rawlings (1995), CV only works when regression coefficient of natural log of error variance as well as regression coefficient of natural log of mean is 2 (Bowman, 2001).

According to Ahmadi and Mollazade (2009), NDVI has a linear relationship with plant population. If plant population increases then NDVI readings increases accordingly. This is due to higher germination which leads to higher ground coverage and higher NDVI reading. Difference in plant absorption and reflection of light largely depends on the relative amount of plant canopy and background soil present in the field of view (Stanhill, 1972). During sensing, the sensor field of view captures reflected light from plant canopy as well as bare soil (in absence of plant). Plant canopy results higher NDVI value as compared to bare soil. Coefficient of variation among these NDVI readings measures variability among the captured NDVI readings taken from experimental plots. Therefore, CV of NDVI readings can be used to measure population stand variability in the field (Arnall et al., 2006). If the CV value is high, variability among crop stand is also high and vice-versa (Lukina et al., 2000). According to Arnall et al. (2006), response $index_{harvest}$ ($RI_{harvest}$) can be calculated accurately when CV is integrated with RI_{NDVI} in winter wheat. Sometimes, the distribution of the plant is heterogeneous but the size of the individual plants is bigger. During sensing, sensor captures reflectance readings from soil as well as plant canopy and because of the bigger plant size, the pooled NDVI is high. When N recommendation is done based on the NDVI reading, it can potentially overestimate the amount of N to be applied. In addition, the rate of application throughout that plot should not be uniform.

Generally, denser plant stand should receive higher N rates than thinner plant stand or bare soil. Incorporation of CV in an N recommendation model gives the idea about the distribution of plant stand in a plot and recommends a precise site specific N rate depending on that information. According to Arnall et al. (2006), optimization of N fertilizer application, reduction of input cost, and N loss from crop field can be done with the application of CV among NDVI. In addition, a study conducted by Martin et al. (2007) revealed that plant biomass is negatively correlated with CV. Measurement of CV among NDVI also gives better estimation of plant biomass yield as compared to the results produced by NDVI alone (Martin et al., 2007).

Keeping all these conditions in mind, the overall goal of this experiment was to **e**valuate the use of CV of NDVI readings to estimate stand population of different cane varieties as affected by planting scheme (billets vs. whole stalk).

4.2 Specific Objectives

1. To establish the relationship between CV of NDVI readings and stand population of cane planted using whole stalk and billets.
2. To relate early plant population stand with millable stalk production; and
3. To evaluate if there is any effect of varietal characteristics on yield.

4.3 Materials and Methods

4.3.1 Site location and layout

This study was established at two sites, site A (30º15'47"N 91º05'54"W) and site B (30º16'08"N 91º06'10"W), at the Louisiana State University AgCenter Sugar Research Station in St. Gabriel, Louisiana. Before planting, the fields were divided into four quadrants and flagged. Sixteen random core samples per quadrant for a total of 64 samples per plot were collected. The samples were dried and ground and extracted with Mehlich 3 solution (Mehlich, 1984) to determine multi element concentration. Carbon:nitrogen (C:N) ratio was determined using CN

analyzer (Model: Vario el cube; Manufacturer: Elementar). Site A was acidic with pH 5.5 while site B was slight acidic with pH 6.04. Site B had a slightly higher CN ratio of 7:1 compared to site A with 6:1. The background soil information is described in Table 4.1.

Table 4.1: Chemical properties of composite soil samples collected from site A and B.

Properties	**A**	**B**	**Interpretation**
Soil type/texture	Commerce silt loam		
Soil classification	fine-silty, mixed, nonacid, thermic Aeric Fluvaquent		
pH	5.5	6.04	
C:N ratio	6:1	7:1	
Phosphorus, mg kg^{-1}	34	41	High
Potassium, mg kg^{-1}	170	219	High
Magnesium, mg kg^{-1}	458	572	Very high
Sulfur, mg kg^{-1}	10.8	11.7	Medium
Copper, mg kg^{-1}	3.5	4.7	Within critical limit

4.3.2 Layout of site A and site B

There were two rows in each plot and the length of the rows was 12 meter. Spacing between rows was 1.83 m. The length of the alley in rows between plots was 1.52 m. The treatments included six cane varieties (four energy canes and two sugarcanes) and two types of planting materials (whole stalk and stalk sections or billets). The four energy cane varieties were Ho 02-113, US 72-114, Ho 06-9001, and Ho 06-9002 while the two sugarcane varieties were L 01-299, and L 03-371. Treatments were arranged in a split plot in randomized complete block design where planting material was designated as the main plot and variety as sub-plot with four replications.

4.3.3 Planting and fertilization

Planting was done on September 13 to 14, 2012 for site A and October 14, 2013 for site B. Two types of planting materials were used in this experiment: whole stalks and billets. Billets were prepared by cutting cane stalks with a combine harvester. In this case, the green cane was

harvested without burning the leaves. The harvested cane stalks were then chopped by the machine while passing through it. A fan was generally attached to it which blew air in such a way that the lighter leaves and tops could be separated from the mature heavier weight stalks. The average length of billets was about 55 cm having an average of three buds per billets. Beds with 1.8 m width were opened where 5 to 6 running billets were placed. For whole stalk planting, stalks of different cane varieties were manually cut with cane knives. Three whole stalks were placed side by side in the furrow with 6-8 cm overlapping. The average length of whole stalks were 1.81, 1.82, 1.97, 1.92, 2.07, and 2.15 m for the cane varieties Ho 02-113, Ho 06-9001, L 01-299, L 03-371, US 72-114, and Ho 06-9002, respectively. The average number of buds on each whole stalk was 13, 13, 12, 13, 12 and 11 for Ho 02-113, Ho 06-9001, L 01-299, L 03-371, US 72-114, and Ho 06-9002, respectively. The whole stalks were planted manually to ensure uniform distribution of planting materials. Table 4.2 is showing the bud density for different planting material during energy cane planting.

Table 4.2. Number of buds present per square meter area for different cane varieties under different planting materials.

Planting materials	Bud number m^{-2}					
	Ho 02-113	Ho 06-9001	L 01-299	L 03-371	US 72-114	Ho 06-9002
Billets	11	11	8	8	8	8
Whole stalks	12	12	10	11	12	10

4.3.4 Vegetation indices used for calculating CV

The following vegetation indices were used to calculate CV and relate with the plant population stand:

$$\mathrm{NDVI} = \frac{\rho_{\mathrm{NIR}} - \rho_{\mathrm{red}}}{\rho_{\mathrm{NIR}} + \rho_{\mathrm{red}}}$$

$$\mathrm{NDVI}_{710} = \frac{\rho_{\mathrm{NIR}} - \rho_{710}}{\rho_{\mathrm{NIR}} + \rho_{710}}$$

$$\mathrm{NDVI}_{735} = \frac{\rho_{\mathrm{NIR}} - \rho_{735}}{\rho_{\mathrm{NIR}} + \rho_{735}}$$

4.3.5 Data collection and processing

Plant shoot population counts were made 4 weeks after planting and at approximately with four week intervals until freezes occurred for site A in 2012. Plant population count started in the month of March at 4 weeks interval until tillering stage for site B in 2014. Plant population sensing using 4-band GreenSeeker® was done only in 2014 for site B because of the unavailability of this sensing unit during 2013. Tiller count was initiated again after recommencement of crop growth (for site A) in the month of May until maximum tillering stage at one week interval. Stalk counts were made in the month of July. Tiller count was started in the month of May at one week interval till maximum tillering stage for site B also.

GreenSeeker® is an active sensor; it emits light and can be used irrespective of the presence of sun light. It emits visible and near- infrared light and collects the reflected visible and near infrared light from the crop canopy. Normalized difference vegetation index was measured by called 2- and 4–band GreenSeeker®. In 2-band GreenSeeker® the wavelengths are 650 nm and 770 nm while, in 4-band GreenSeeker®, the wavelengths are 660, 710, 735, and 770 nm. It provided data in the form of average NDVI as well as individual NDVI readings of the plot. The NDVI data ranges from 0 to 1 (the healthier the plant, the higher is the NDVI value). The NDVI readings were used to analyze CV. Coefficient of variation among NDVI readings with in a plot was calculated using Microsoft Excel 'Macro' (Microsoft, Seattle, WA). Then the CV of NDVI and cane population stand relationship were evaluated by correlation regression analysis.

Millable stalk population was counted manually at grand growth stage which approximately reached by cane within the month of July. Relation of millable stalk population

with plant and tiller population were also evaluated. Plant canopy characteristics were also measured during that time. These included plant height, leaf length, length where leaf bent (hypotenuse), angle of the leaves with the stem (canopy openness), leaf blade width, and stem width. These data were used to evaluate varietal effects on spectral reflectance. For canopy characters estimation, five plants were selected randomly from one plot and all the parameters were measured from those selected plants. Plant height was measured using a meter stick from base to the first visible node at the top i.e. collar region. Leaf length was measured from the base of the leaf blade to the tip. Leaf width was measured at five random places for each leaf and averaged it. Leaf angle was measured using a protractor. Stem width measurement was done with slide calipers at five random spots. Distance from stem where the leaf bent was measured using a meter stick. Measurement of hypotenuse was done from the leaf angle and the distance from stem where leaf bent.

Analysis of variance was done using PROC MIXED with SAS 9.3 (SAS Institute, 2012) to evaluate the effect of planting materials on cane population, tiller population, and millable stalk population. Mean separation was done by Tukey–Kramer post-hoc test. Regression analyses were done using PROC REG to evaluate the relation between plant population and CV of NDVI readings computed from reflectance readings at different wavelengths. Principal component analyses were done to identify the parameters that can separate the varieties and find out if there is any varietal effect on canopy spectral reflectance. Homogeneity of variance was tested by Bertlett method (Bartlett, 1937).

4.4 Results and Discussion

Effect of different planting materials on plant population, tiller number, and stalk population were presented in Table 4.3. Planting materials had significant effect on cane stand in 2013 plant cane only (*P*<0.05).

Table 4.3. Effect of planting materials on plant population, tiller number, and stalk population of canes.

Parameters	Site A 2013 plant cane[1]		Site B 2014 plant cane[1]		Site A 2014 first ratoon[1,2]	
	Billets-planted	Whole stalk-planted	Billets-planted	Whole stalk-planted	Billets-planted	Whole stalk-planted
Plant population '000 ha^{-1}	91_a	60_b	48_a	51_a	-	-
Tiller population '000 ha^{-1}	411_a	398_a	286_a	308_a	366_a	331_a
Stalk population'000 ha^{-1}	219_a	181_b	158_a	169_a	151_a	158_a

[1]Same letter within row and site year indicate no significant differences between the treatment means based on Tukey–Kramer post-hoc test (*P* = 0.05).
[2]First ratoon in 2014 did not have the information. First ratoon germinated as tillers and there was only tiller population for that plot.

In 2013 plant cane, billet-planted plots produced significantly higher (*P* < 0.05) plant population (1.52 times) than the whole stalk-planted plots. Whole stalk planting may produce poor stand due to apical dominance (Orgeron, 2007). Plant hormones like auxin suppress the germination of the lower buds and the degree of suppression was increasing from top to bottom (James, 2004; Orgeron et al., 2007). This might be the reason of lower plant population stand for whole stalk-planted cane in 2013. During tiller production in spring of 2013 in site A, the difference in tiller population affected by two planting materials reduced to 1.03 times and there was no significant difference (*P* > 0.05) between them. As sugarcane is tillering grass plant, it has a tendency for producing profuse tiller which fill up the existing gap between plants for both planting materials. Hence, the extent of difference in tiller population between different planting

materials was reduced and became statistically similar. All the tillers produced by cane plants did not survive for both planting methods due to competition for nutrient and water as well as due to mutual shading. According to Sangplung and Rosario (1978), sugarcane produced maximum tiller population at 4 months after planting. Later on there was decrease in plant population due to the death of late germinated tillers. Billet-planted cane produced 1.21 times higher millable stalk count than whole-stalk planted cane.

In 2014, both planting materials produced similar plant population, tiller population, and stalk numbers. A similar trend was found for the first ratoon. Billet-planted cane not only failed to produce higher plant population than the whole-stalk planted cane, it produced slightly lower population stand. This can be attributed to severe cold temperature in 2014 that affected the billet-planted cane more adversely than whole stalk-planted cane. In harsh winter, the tendency of the above-ground biomass of cane is to dry up leaving the cane dormant until favorable temperature prevails. At this dormant stage crop utilizes the reserved food present in the stalk for survival. The high food reserve in whole stalk as compared to billet typically results in better growth and performance of whole stalk-planted cane in adverse conditions. Hence, under adverse weather condition whole stalk is a better planting material than billets. In addition to this, some studies showed that the injury made on both sides of billets resulted in higher damage by stalk rot in billets-planted cane than whole stalk-planted cane (Yin et al., 1996; Hoy, 2001; Hoy et al., 2004). Cutting whole stalks into billets increases the surface area expose to pathogen attack as compared to the whole-stalks.

There were significant difference in the early-season plant population, tiller number, and cane stalk number observed among varieties ($P < 0.05$) (Table 4.4). For 2013 and 2014 plant cane, there was no effect of varieties on early plant population and tiller number, respectively.

Table 4.4. Effect of planting materials and varieties on early plant population, tiller numbers, and cane stalk population.

Varieties	Early plant population[1]	Tiller number[1]	Cane stalk number[1]
2013 Plant cane site A			
Ho 02-113	84 $_{a}$	435 $_{ab}$	214$_{ab}$
Ho 06-9001	65$_{a}$	471$_{a}$	226$_{a}$
L 01-299	73$_{a}$	362$_{bc}$	168$_{ab}$
L 03-371	75$_{a}$	324$_{c}$	163$_{b}$
US 72-114	91$_{a}$	429$_{abc}$	206$_{ab}$
Ho 06-9002	67$_{a}$	408$_{abc}$	224$_{a}$
2014 First ratoon cane site A			
Ho 02-113	-	384$_{abc}$	173$_{ab}$
Ho 06-9001	-	399$_{ab}$	147$_{ab}$
L 01-299	-	236$_{bc}$	119$_{b}$
L 03-371	-	194$_{c}$	118$_{b}$
US 72-114	-	392$_{ab}$	164$_{ab}$
Ho 06-9002	-	550$_{a}$	235$_{a}$
2014 Plant cane site B			
Ho 02-113	73$_{a}$	425$_{a}$	198$_{a}$
Ho 06-9001	30$_{b}$	376$_{a}$	192$_{a}$
L 01-299	67$_{a}$	315$_{a}$	125$_{bc}$
L 03-371	52$_{ab}$	272$_{a}$	111$_{c}$
US 72-114	47$_{ab}$	444$_{a}$	188$_{a}$
Ho 06-9002	27$_{b}$	322$_{a}$	173$_{ab}$

Same letter within column and year by site indicate no significant differences between the treatment means based on Tukey–Kramer post-hoc test ($P = 0.05$).

Favorable temperature during germination period in 2012 might be the reason for the lack of varietal effect on early-season plant population in 2013 plant cane. In contrast, due to cold damage in 2013 winter, the germination and early-season plant population of 2014 plant cane suffered. This has caused varietal differences to occur such that Ho 02-113 and L 01-299 produced significantly higher early-season plant population than Ho 06-9001 and Ho 06-9002 ($P < 0.05$). These two varieties had the lowest stalk weight among the other varieties which resulted in poor cane germination (Table 4.5). Smit (2010) found out that sugarcane germination was affected by temperature and when the temperature was less than 20ºC, the germination rate was low. Giardina et al. (2013) showed that varieties can affect the amount of seed cane germination.

In the present study, energy cane varieties produced numerically higher number of tiller and millable stalk than sugarcane varieties. The stalk parameters are summarized in Table 4.5.

Table 4.5. Stalk parameters of different cane varieties.

Varieties	Stalk parameters			
	Node number[1]	Weight (kg)[1]	Diameter (mm)[1]	Length (m)[1]
Ho 02-113	16±3.19	0.38±0.13	13.82±2.20	2.43±0.31
US 72-114	18±2.38	0.49±0.14	14.60±1.49	2.70±0.33
L 01-299	17±2.58	0.89±0.25	19.67±2.19	2.62±0.24
L 01-371	17±2.06	0.89±0.28	21.03±2.59	2.18±0.30
Ho 06-9001	18±2.78	0.32±0.08	12.92±1.53	2.84±0.34
Ho 06-9002	17±2.74	0.29±0.06	12.83±1.16	2.78±0.32

[1]Values are presented as mean±standard deviation.

Both sugarcane varieties L 01-299 and L 01-371 had higher stalk weight than the energy cane varieties due to thicker stalks. Stalks were taller for varieties Ho 06 9001, US 72-114, and Ho 06-9002 and had higher node numbers, while shorter variety Ho 02-113 had shorter stalk and lower node count (Table 4.4).

Table 4.6. Test of homogeneity of variance for different planting materials, varieties and years on early plant population, tiller numbers, and cane stalk population.

Sensing time	Parameters	*P*-values across early plant, tiller, or stalk population	
		Billets-planted cane	Whole stalk-planted cane
1	Crop year	0.596	0.361
2		0.465	0.134
3		0.006	0.030
4		0.001	<0.0001
1	Planting materials	<0.0001	
2		0.0035	
3		0.3011	
4		0.9269	
1	Variety	0.2821	0.0529
2		0.5448	0.2112
3		0.0195	0.0577
4		0.0006	<0.0001

Homogeneity of variance was tested using Bartlett method for different varieties, site years, and planting materials (Table 4.6). Plant population for varieties and crop years were heterogeneous and cannot be pooled at third and fourth sensing. In addition, plant populations for planting materials were heterogeneous for first and second sensing. The initial heterogeneity in population stand during early-season sensing was due to difference in early population stand. The heterogeneity in crop year and varieties was due to the ability of the different varieties to produce tillers at different crop stage (i.e. plant cane and ratoon cane).

When the CV and plant populations were plotted separately by crop year, the r^2 values slightly improved for all varieties. However, the relation was not significant (P>0.05) due to lack of data points (8 data points). Conversely, separating by planting materials at early growing stage did not improve the r^2 (range 0.06 to 0.15) values at all but the relation turned out to be significant.

Although the variance was not homogeneous across crop age, varieties, and planting materials, the data were pooled to establish the relationships between CV of NDVIs (calculated using reflectance from different wavebands) and cane population. Both planting materials and varieties showed differences in plant population, tiller number and stalk population. One aspect of this study was to evaluate if the CV of sensor readings can detect the variability in cane stand accordingly. Also, another aim of pooling the data was to incorporate all the weather, crop age, and site variability to develop a generalized model applicable for all growing season. Arnall et al. (2006) also pooled all the sites and years before plotting CV of NDVI against plant population. Furthermore, the critical CV was identified using Cate-Nelson model.

A very weak relation exists between CV of $NDVI_{red}$ captured by 2-band GreenSeeker® and plant population in whole stalk-planted cane with r^2 value of 0.03 (Fig. 4.1 A).

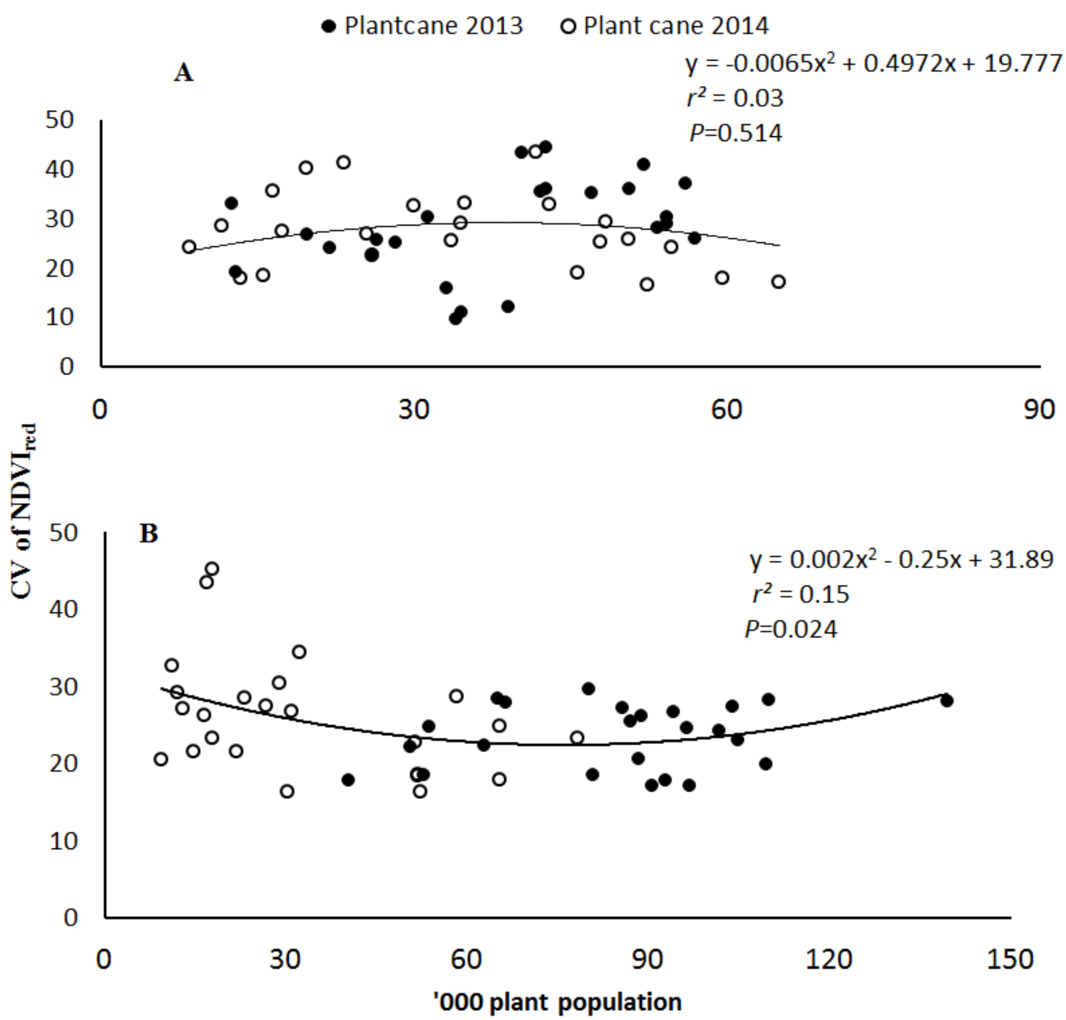

Figure 4.1. Relation between plant population and CV of $NDVI_{red}$ collected using 2-band GreenSeeker® one month after planting for whole stalk-planted (A) and billet-planted (B) cane.

At this stage, plant population and canopy structure were too small to produce any strong relationship with CV of $NDVI_{red}$. A better relation was observed in billet-planted cane (Fig. 4.1 B) with r^2 value of 0.15. As the whole stalk-planted cane produced lower and more dispersed plant population compared to billet-planted cane, the strength of relation was weaker.

Plant population at two months after planting produced better relation with CV of $NDVI_{red}$ collected by 2-band GreenSeeker® (Fig. 4.2). At this stage, the shoot population was 1.4 times higher than 1 month crop age.

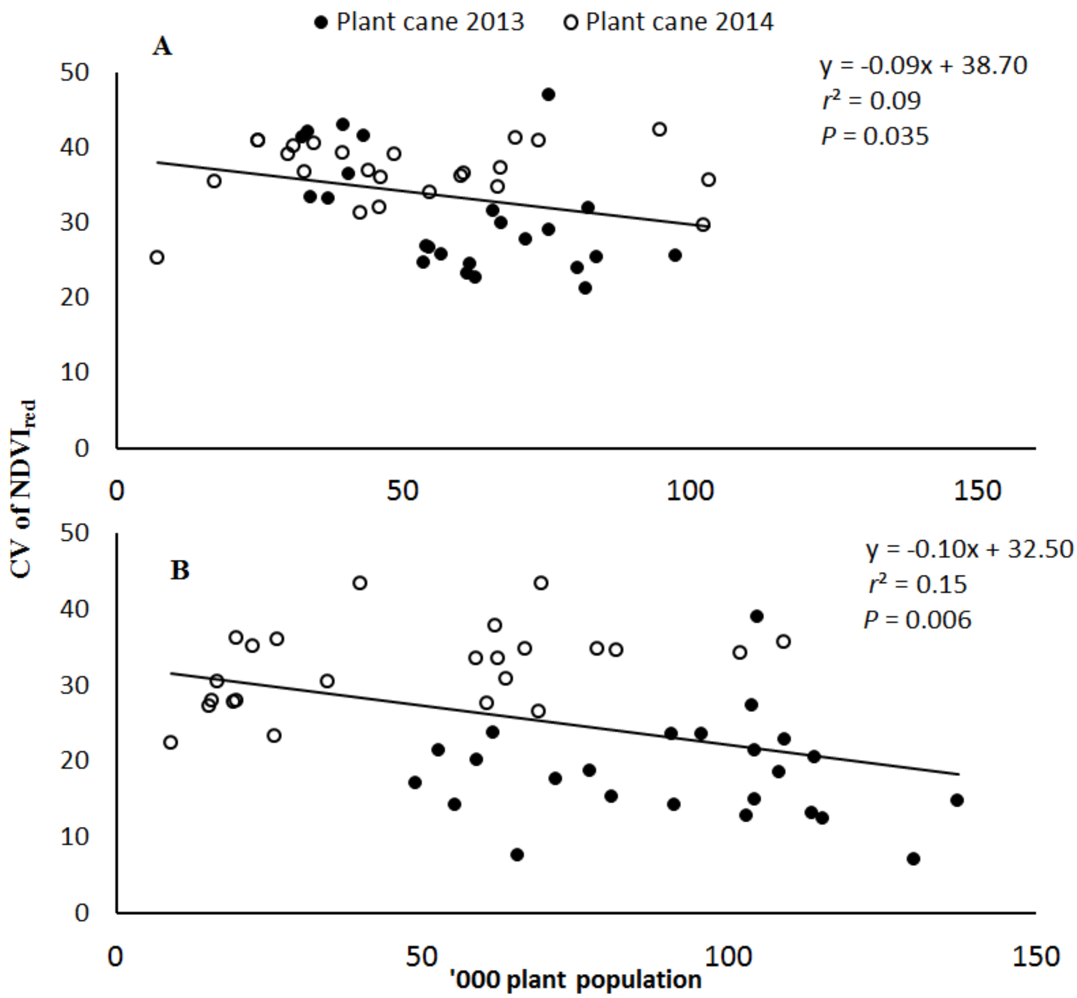

Figure 4.2. Relation between plant population and CV of $NDVI_{red}$ collected using 2-band GreenSeeker® two month after planting for whole stalk-planted (A) and billet-planted (B) cane.

Due to denser plant stand, billet-planted cane produced better relationship (r^2= 0.15) with CV of NDVI and plant population than the whole stalk-planted cane (r^2=0.09). Both of the cases the relationship was negatively linear.

Early tiller population in the spring showed the strongest relationship with CV of $NDVI_{red}$ collected by 2-band GreenSeeker® among all other sensing stages (Fig. 4.3).

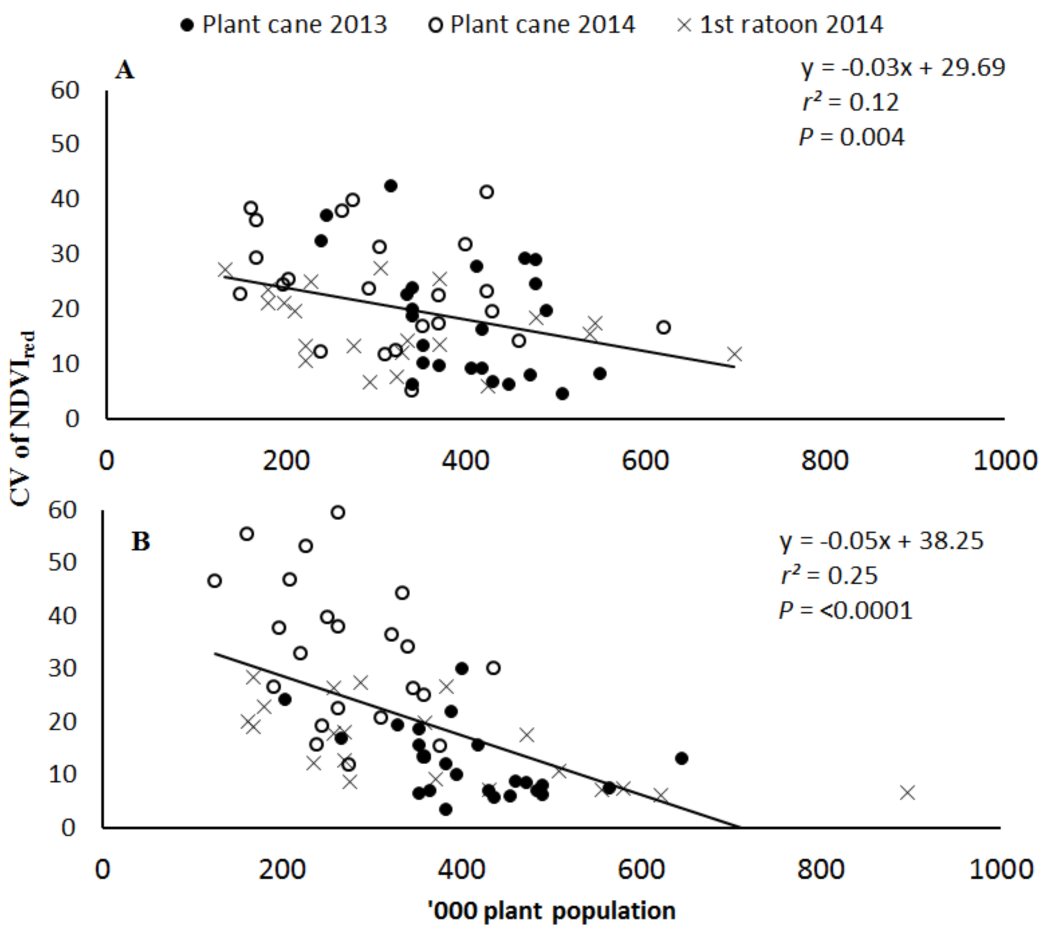

Figure 4.3. Relation between plant population and CV of $NDVI_{red}$ collected using 2-band GreenSeeker® at early tillering stage for whole stalk-planted (A) and billet-planted (B) cane.

At early tillering, population count was increased by six times over the plant population at two months crop age. As a result, the canopy coverage became wider which made the population stand appeared more uniform. Cane produced the strongest relationship between population stand and CV of $NDVI_{red}$ at this stage and the relation pattern was linear. At this stage also billet-planted cane showed a stronger correlation between CV of NDVI and tiller population than the whole stalk-planted cane.

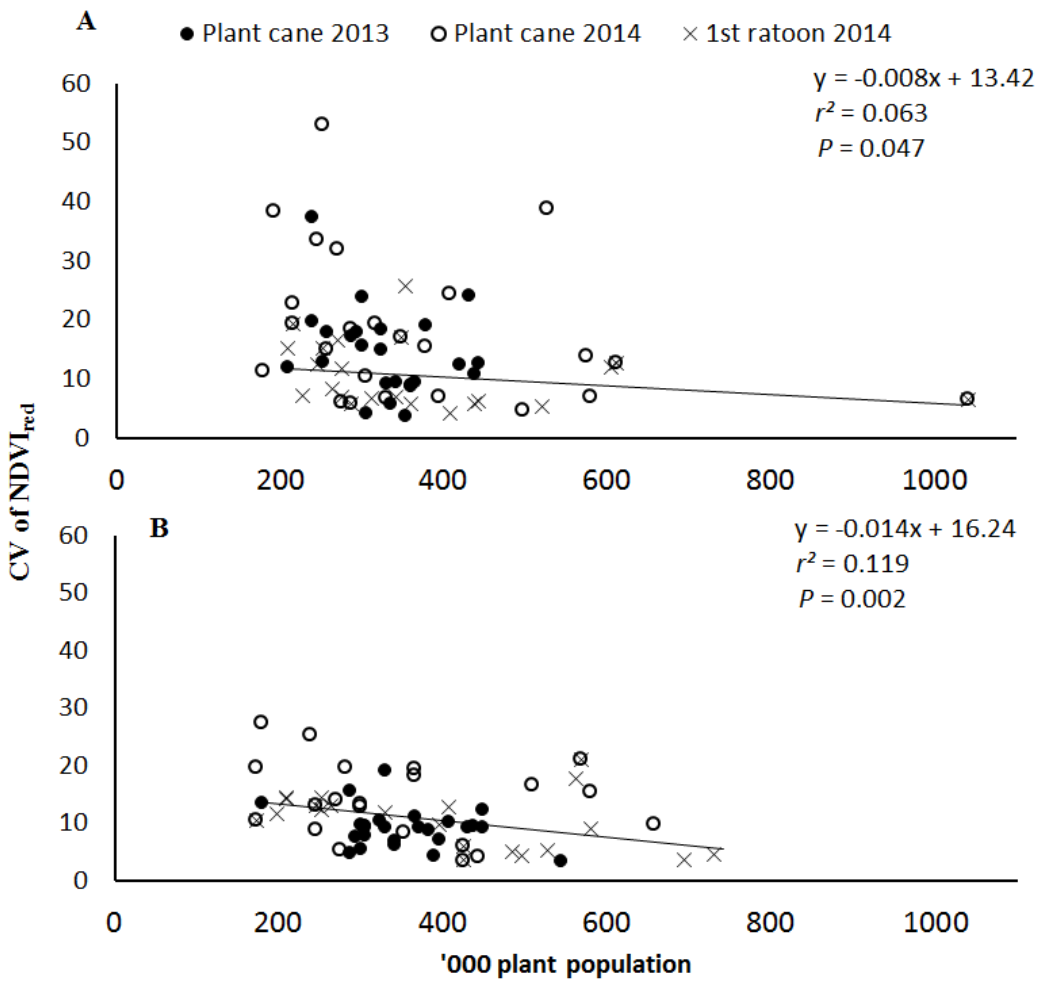

Figure 4.4. Relation between plant population and CV of $NDVI_{red}$ collected using 2-band GreenSeeker® at tillering stage for whole stalk-planted (A) and billet-planted (B) cane

At maximum tillering stage, the strength of relation was weaker than the early tillering stage (Fig. 4.4). This might be due to the regular ground operation during this period which resulted in cutting and burying some plants from the field. Due to higher plant coverage, the NDVI value was high and CV value was low. It was also noted that plant population was also lower than the early stage. In addition, the amount of tillers produced at early stage of growth did not all contribute to the stalk population at harvest. There was death of tillers due to mutual shading, competition for space and nutrient uptake. Hence there was 1.2 times less population than the early tillering stage, which impacted the strength of relation adversely. Sangplung and

Rosario (1978) found that the tiller production of cane reached maximum until 4 months and after this stage, a decrease in tiller number was observed due to the death of smaller tiller. According to Martin et al. (2007), early-season growth stage of corn produced weaker relationship between CV of NDVI and change of plant spacing as compared to the intermediate growth stages such as V7 to V9. He also found that at V10 growth stage of corn sensor could not able to detect the change of plant spacing due to closer canopy structure.

A similar trend was observed for the data collected by 4-band GreenSeeker® (Table 4.7). Coefficient of variation was negatively correlated with tiller population as the slope was negative in most equations.

Table 4.7. Relation between cane population (x) and CV of different NDVIs collected using 4-band GreenSeeker® under two different planting materials.

Sensing time	Whole stalk-planted cane			Billet -planted cane		
	r^2	*P*-value	Equation[1]	r^2	*P*-value	Equation[1]
1	0.23	0.018	CV_{red} = 0.09 x + 10.41	0.26	0.011	CV_{red} = 0.06x + 8.02
	0.005	0.735	CV_{710} = 0.06x + 2.14	0.01	0.602	CV_{710} = 0.03x + 38.62
	0.0007	0.906	CV_{735} = -0.007x + 3.36	0.00	0.711	CV_{735} = 0.02x + 32.64
2	0.003	0.816	CV_{red} = -0.01x + 34.03	0.06	0.244	CV_{red} = -0.06x + 37.85
	0.51	<0.0001	CV_{710} = -0.20x + 63.84	0.03	0.392	CV_{710} = -0.05x + 58.99
	0.07	0.215	CV_{735} = -0.06x + 53.82	0.04	0.371	CV_{735} = -0.03x + 53.58
3	0.16	0.001	CV_{red} = -0.03x + 28.68	0.37	<0.0001	CV_{red} = 0.0001x^2 - 0.13x + 48.76
	0.16	0.001	CV_{710} = -0.20x + 30.69	0.32	<0.0001	CV_{710} = 0.0001x^2 - 0.13x + 52.10
	0.03	0.208	CV_{735} = -0.01x + 29.87	0.24	<0.0002	CV_{735} = -0.03x + 37.41
4	0.03	0.145	CV_{red} = -0.02x + 26.84	0.14	0.002	CV_{red} = -0.04x + 32.07
	0.04	0.133	CV_{710} = -0.02x + 30.64	0.14	0.002	CV_{710} = -0.04x + 36.63
	0.04	0.135	CV_{735} = -0.02x + 31.89	0.12	0.003	CV_{735} = -0.02x + 35.71

[1]CV_{red} = CV of $NDVI_{red}$, CV_{710} = CV of $NDVI_{710}$, CV_{735} = CV of $NDVI_{735}$

At first and second sensing, which was done one and two month crop age, respectively, crop canopy coverage and population count were too small to produce any significant relationship.

At 3rd sensing time i.e. early tillering stage, CV of NDVIs showed stronger correlation

than other sensing time wherein the highest r^2 values (r^2=0.16 and 0.37 for whole stalk- and billets-planted cane, respectively) were obtained at red wavelength. Due to ground operation (harrowing), there was destruction of tillers at 4th sensing time which results weaker relation between tiller count and CV of NDVI at this stage. Overall, CV among $NDVI_{red}$ provided constantly stronger relation than other wavelengths across different sampling time. Similar pattern was observed by Martin et al. (2007) where the CV of NDVI at intermediate growth stage of corn (V7 to V9) produced the strongest relationship with plant population as compared to the early or later growth stage of corn.

A similar trend was observed in relation between millable stalk production and early plant population (Table 4.8). Population count during 3rd and 4th sensing had the strongest relationship with millable stalk production. This indicates that it is better to do plant population monitoring at later growth stage. Millable stalks production largely depended on tiller production than the individual plant germination.

Table 4.8. Relation between cane population (x) and stalk population (SP) under different planting materials.

Sensing time	Whole stalk-planted cane			Billet -planted cane		
	r^2	*P*-value	equation	r^2	*P*-value	equation
1	0.01	0.896	$SP = -0.009x^2 + 0.36x + 171.56$	0.16	0.559	$SP = 0.33x + 146.99$
2	0.02	0.801	$SP = -0.009x^2 - 1.09x + 198.58$	0.15	0.856	$SP = -0.005x^2 + 0.74x + 139.19$
3	0.24	<0.0001	$SP = 0.23x + 89.20$	0.32	<0.0001	$SP = -0.0003x^2 + 0.55x + 30.75$
4	0.25	<0.0001	$SP = -0.0003x^2 - 0.51x + 47.59$	0.30	<0.0001	$SP = -0.0006x^2 – 0.71x + 12.33$

Canopy parameters of cane varieties were summarized in Table 4.9. Harvesting the smallest measured leaf angle, sugarcane variety L 01-371 has a more droopy-leaf canopy

structure relative to the other varieties. Both of the sugarcane varieties had thicker stalks than the energy cane varieties.

Table 4.9. Parameters affecting canopy development in different cane varieties.

Varieties	Canopy parameters					
	Leaf length (cm)[1]	Leaf width (cm)[1]	Plant height (mm)[1]	Hypotenuse (m)[1]	Leaf angle (°)[1]	Early stalk diameter (mm)[1]
Ho 02-113	135±17.00	1.32±0.34	145±19.43	44.01±12.04	65.76±10.27	14.70±3.14
US 72-114	134±19.34	1.39±0.37	152±21.23	45.59±16.73	68.93±9.49	15.19±2.48
L 01-299	128±12.00	1.73±0.38	136±18.29	44.71±15.08	66.95±7.77	19.05±3.98
L 01-371	134±11.66	1.74±0.24	135±19.59	50.67±6.25	58.24±6.77	20.95±2.96
Ho 06-9001	111±36.54	1.35±0.43	157±29.03	60.04±40.91	62.45±14.08	14.10±2.89
Ho 06-9002	108±38.72	1.38±0.35	166±19.35	64.20±41.45	65.16±12.68	14.49±1.94

[1]values are presented as mean±standard deviation.

Energy cane variety Ho 06-9002 was the tallest, while both the sugarcane varieties were shorter. Both sugarcane varieties had wider leaves than energy cane varieties. Hypotenuse determines the openness of the canopy. A longer hypotenuse along with larger angle indicates canopy erectness. Variety Ho 06-9002 produced larger leaf bent length and angle hence has more erect canopy.

Principal component (PRIN) 1 contributed 70% while PRIN2 contributed 16% of the total variability among varieties of cane. Other PRIN contributed negligible amount of variability (Table 4.10). Hence, PRIN1 and PRIN2 were considered. According to Cruz and Carneiro (2006), for an efficient principal component analysis (PCA), the cumulative variability should be 80% for first two PRIN so that it can explain the majority of variation in two axes (Martuscello, 2012). Hypotenuse explained the highest variability among varieties followed by plant height (Table 4.11). Hypotenuse contributed variability to the PRIN1 while plant height contributed the highest to the variability to the PRIN2 and second highest to the PRIN1. There was no effect of NDVI on plant varieties.

Table 4.10. Eigenvalues of covariance matrix of principal component analysis (PCA) for canopy characters and NDVI readings of different cane varieties.

Eigenvalues of the covariance matrix				
Principal components	Eigenvalue	Difference	Proportion	Cumulative
1	1026.55	795.64	0.70	0.70
2	230.91	126.96	0.16	0.85
3	103.95	33.13	0.07	0.93
4	70.82	35.17	0.05	0.97
5	35.64	32.63	0.02	1.00

Table 4.11. Eigenvectors of principal component analysis (PCA) for canopy characters of different cane varieties.

Eigenvectors		
Character	PRIN1	PRIN2
Plant height	0.26	0.93
Leaf angle	0.16	-0.03
Leaf length	0.11	0.12
Hypotenuse	0.94	-0.26
Leaf width	0.00	0.00
Stalk diameter	-0.01	0.19
$NDVI_{red}$	0.00	0.00
$NDVI_{710}$	0.00	0.00
$NDVI_{735}$	0.00	0.00

All energy cane and sugarcane varieties are under the same genus *Saccharum* and their plant structure is similar. Hence, the PRIN1 and PRIN2 graph across varieties did not show any clean separated clusters and there is a lot of overlapping among varieties (Figure 4.5). This is because varieties may vary in more parameters than those analyzed in this objective. Principal component 1 was able to separate between sugarcane varieties L 01-299 and L 03-371. Sugarcane variety L 03-371 had higher hypotenuse with smaller angle and indicated droopy canopy. Ho 02-113 and US 72-114 could be separated by PRIN 1.

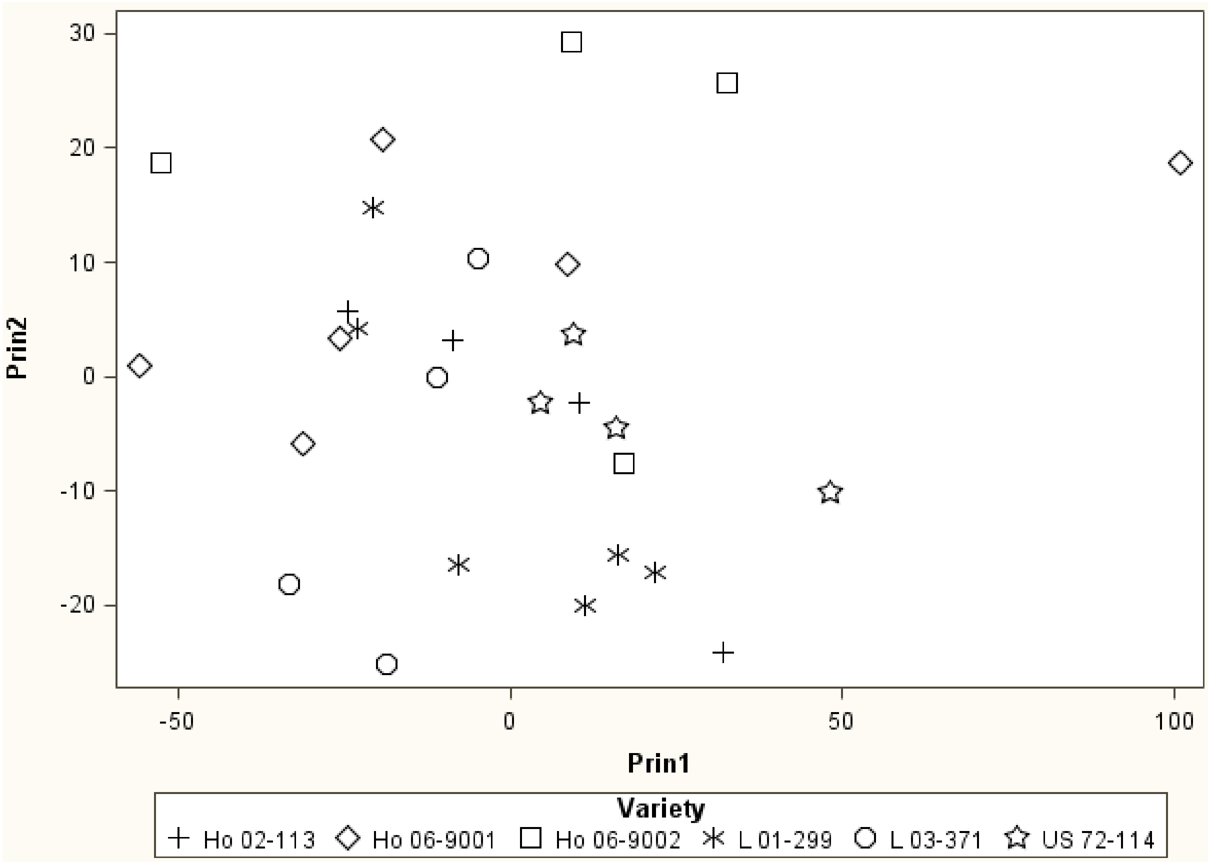

Figure 4.5. Scores of different varieties plotted against PRIN1 and PRIN2.

Energy cane variety US 72-114 had longer hypotenuse and larger leaf angle than Ho 02-113 which indicated that the former had more erect canopy. Two sugarcane varieties were separated from energy cane varieties by PRIN2. This was because sugarcane varieties were shorter than energy cane varieties. The tallest variety Ho 06 9002 were separated from others by PRIN 2.

The planting materials had no effect on yield for plant cane 2013 and 2014 but showed significant effect on yield for 2014 first ratoon crop ($P<0.05$) (Table 4.12). Furthermore, there was an interaction effect between planting materials and varieties on fiber yield only present in 2014 first ratoon cane.

Table 4.12. Summary of analysis of variance (ANOVA) showing level of significance for different sugar parameters as effected by different varieties and planting materials of cane.

Year	Crop	Treatments	*P*-value				
			Brix	TRS	Fiber	Sucrose	Yield
2013	Plant cane	Variety	0.155	<0.0001	<0.0001	<0.0001	0.0247
		Planting material	0.4296	0.0792	0.9185	0.1062	0.9143
		Variety*Planting material	0.544	0.9276	0.8495	0.8953	0.1469
2014	First ratoon	Variety	<0.0001	<0.0001	<0.0001	<0.0001	0.0204
		Planting material	0.2776	0.2284	0.4556	0.2268	0.0208
		Variety*Planting material	0.5777	0.8941	0.0057	0.8437	0.2189
2014	Plant cane	Variety	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		Planting material	0.2133	0.0968	0.2548	0.1132	0.9635
		Variety*Planting material	0.9983	0;9602	0.1394	0.9839	0.2813

Table 4.13. Main effect of different planting materials on cane tonnage and interaction effect of planting material and variety on fiber production.

Treatment[1]	Parameters	Estimate[2]
Effect of planting materials on cane tonnage for first ratoon 2014		
Whole stalk	Cane tonnage (t ha^{-1})	65.81_{a}
Billet		57.26_{b}
Interaction effect of planting materials on fiber content for first ratoon 2014		
Ho 06-9002 x whole stalk	Fiber content (%)	23.86_{a}
US 72-114 x billet		23.10_{ab}
Ho 06-9001 x whole stalk		22.95_{ab}
Ho 02-113 x billet		21.83_{ab}
Ho 02-113 x whole stalk		21.81_{ab}
Ho 06-9001 x billet		21.69_{ab}
Ho 06-9002 x billet		21.01_{ab}
US 72-114 x whole stalk		20.88_{b}
L 01-299 x billet		12.77_{c}
L 01-299 x whole stalk		12.21_{c}
L 01-371 x whole stalk		11.24_{c}
L 01-371 x billet		11.05_{c}

[1]Whole stalk = whole stalk-planted cane, Billet = billet-planted cane.
[2]Same letter within column and parameter indicate no significant differences between the treatment means based on Tukey–Kramer post-hoc test (P = 0.05).

Hence, the effect of planting material on cane tonnage and the interaction effect between planting materials and varieties on fiber yield for 2014 first ratoon cane are presented in Table

4.13. For first ratoon in 2014, whole stalk-planted cane produced significantly better cane tonnage than billet-planted cane. Overall, both of the sugar cane varieties produced significantly lower fiber content as compared to energy cane varieties irrespective of planting materials. However, interaction effect of planting materials across varieties did not show any clear effect on fiber production

Both sugarcane varieties performed better than energy cane varieties in terms of cane yield and sugar quality (Table 4.14).

Table 4.14. Effect of different varieties on cane tonnage and sugar quality.

Year	Crop year	Variety	Cane yield (t ha^{-1})[1]	Total recoverable sugar[1] (kg t^{-1})	Brix[1] (%)	Fiber[1] (%)	Sucrose (%)[1]
2013	Plant cane	Ho 02-113	67.44$_{b}$	77.93$_{b}$	Ns	20.28$_{a}$	12.14$_{b}$
		US 72-114	50.92$_{c}$	72.25$_{b}$	Ns	23.03$_{a}$	11.07$_{b}$
		Ho 06-9001	29.81$_{d}$	73.80$_{b}$	Ns	23.32$_{a}$	11.65$_{b}$
		Ho 06-9002	35.84$_{d}$	67.68$_{b}$	Ns	24.4$_{a}$	11.06$_{b}$
		L 01-299	86.21$_{a}$	120.32$_{a}$	Ns	13.10$_{b}$	16.63$_{a}$
		L 03-371	63.88$_{bc}$	131.51$_{a}$	Ns	10.47$_{b}$	18.02$_{a}$
2014	First ratoon	Ho 02-113	56.80$_{bc}$	69.86$_{bc}$	16.57$_{bc}$	21.82$_{a}$	11.31$_{bc}$
		US 72-114	47.12$_{c}$	43.94$_{d}$	13.55$_{d}$	21.99$_{a}$	8.02$_{d}$
		Ho 06-9001	42.26$_{c}$	51.42$_{cd}$	16.03c$_{d}$	22.32$_{a}$	9.16$_{cd}$
		Ho 06-9002	47.48$_{c}$	68.51$_{cd}$	16.54b$_{c}$	22.44$_{a}$	11.20$_{bc}$
		L 01-299	86.69$_{a}$	92.93$_{ab}$	18.02$_{ab}$	12.49$_{b}$	13.82$_{ab}$
		L 03-371	78.09$_{ab}$	103.71$_{a}$	19.09$_{a}$	11.15$_{b}$	15.18$_{a}$
2014	Plant cane	Ho 02-113	70.66$_{b}$	58.10$_{c}$	14.45$_{c}$	21.36$_{a}$	9.53$_{c}$
		US 72-114	69.29$_{b}$	31.62$_{e}$	11.72$_{d}$	20.99$_{a}$	6.27$_{e}$
		Ho 06-9001	36.17$_{c}$	47.51$_{cd}$	13.67$_{c}$	19.95$_{a}$	8.31$_{cd}$
		Ho 06-9002	29.41$_{c}$	44.71$_{d}$	13.35$_{c}$	21.47$_{a}$	7.96$_{d}$
		L 01-299	103.56$_{a}$	90.09$_{b}$	16.86$_{b}$	10.82$_{b}$	13.20$_{b}$
		L 03-371	89.96$_{a}$	106.06$_{a}$	18.73$_{a}$	10.87$_{b}$	15.23$_{a}$

[1]Same letter within column indicate no significant differences between the treatment means based on Tukey–Kramer post-hoc test (P = 0.05).

However, energy cane varieties produced significantly higher fiber yield than sugarcane varieties. As a hybrid of wild cane, energy cane contains more fiber than sugarcane cane which can be utilized for production of biofuel (Alexandar, 1985; Matsuoka, 2014). The highest cane

tonnage was produced by variety L 01-299 across all the crop age. Mostly sugarcane varieties produced significantly higher cane tonnage, total recoverable sugar, and sucrose content than energy cane varieties. Sugar content is negatively related with fiber content (Brown, 1965; Ramdoyal and Badaloo, 2002). Variety L 03-371 produced the highest sucrose content than all the varieties.

Among energy cane varieties, Ho 02-113 produced consistently higher cane tonnage, recoverable sugar, brix value, and sucrose content but produced low fiber content. Variety Ho 06-9002 had the highest fiber content among all the varieties. Planting material had no effect on cane tonnage (except 2014 first ratoon cane), sucrose yield, and sugar quality ($P > 0.05$).

4.5 Conclusions

Overall, the performance of planting materials depends on the weather conditions. Billet-planted cane produced higher shoot population and stalk count than whole stalk-planted cane under favorable conditions. However, under adverse conditions, both-planting materials produced similar shoot population and stalk number. So, both the planting materials can be used efficiently for cane production in tropical areas, however, for temperate regions, whole stalk-planted cane is a better choice because of more food reserve and less exposed surfaces for pathogen attacks. Results also showed that due to low germination and scattered distribution, strength of relation between CV of NDVIs and plant population in whole-stalk planted cane was weaker than billets. Overall, CV of $NDVI_{red}$ produced better correlation than other wavebands across crop age and sensing time. In both 2- and 4-band GreenSeeker® evaluations, the early tillering stage plant population produced a higher correlation than other stages. Therefore, the early tillering stages are the most optimum time to sense the crop canopy and to estimate plant population more precisely. Bigger plants with heterogeneous distribution produces higher

average NDVI and N recommendation, but the application of N throughout the plot will not be uniform. So, the incorporation of CV among NDVI component can be used as a beneficial tool for more precise site specific N recommendation. The r^2 values in this study were not high enough to recommend any model. According to the findings of the study, vegetation indices were not affected by different varieties. Hence, any of the vegetation indices can be equally applicable for different varieties. More study is needed to confirm this finding. Overall, energy cane varieties were taller than sugar cane varieties. Planting materials had no effect on sugar quality and sucrose production. Sugarcane varieties produced significantly higher cane tonnage and sugar yield as compared to all the energy cane varieties. Due to higher sugar content, it is easier to obtain bioethanol from those sugarcane varieties. But, due to potential competition between food and feedstock production and price increase of cane sugar, use of energy cane is a good alternative for bioethanol production. As energy cane varieties produced significantly higher amount of fiber than sugarcane varieties, it can serve as a second generation biofuel crop. In this experiment Ho 06-9002 numerically produced highest fiber among all energy cane varieties and can be used to obtain more bioethanol.

4.6 References

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Chapter 5. Conclusions

The established method of estimating crop yield using canopy spectral reflectance is to correlate the yield at harvest with VI readings collected at an early stage of crop growth. This study was conducted to find out if early-season biomass of energy cane was related with early season canopy spectral reflectance collected at the same time. Also, to evaluate if any similarity existed between the biomass yield pattern at early and at harvesting crop stage. Two planting materials, billets and whole stalks, were used for cane production and their effect on population stand and yield was also evaluated.

This experiment showed that early-season biomass yield and VI were positively correlated across sampling season for all crop ages. Among different sampling times, overall, four WAN was identified as an appropriate time to collect data as the relation of early-season biomass and VI at this time was stronger than the other two sampling times. Quadratic model could predominantly explain the relationship between early-season biomass and VI collected at the same time. Visible wavelengths showed negative correlation while NIR showed positive correlation with plant biomass yield. This was because chlorophyll absorbs more in visible light and higher biomass leads to higher chlorophyll concentration and more absorption of visible spectrum of light. Internal cellular scattering of leaf produced a higher reflectance peak at NIR wavelength. Hence, more biomass leads to more layers of cells and a higher degree of scattering and reflectance: wavelength at 690 nm produced maximum negative correlation while 740 nm produced maximum positive correlation. Overall, reflected red light performed better in producing stronger relation with plant biomass yield. Relation between REP with cane biomass production was highly variable depending upon variety, crop age, and sampling time. Based on

these findings, $NDVI_{red}$ is still useful for biomass estimation of energy cane although further research is needed for refinement.

Similarity exists in the biomass production and N uptake pattern between early stage and harvesting. Closer N response pattern to that at harvest were produced at 4 and 5 than the 3 WAN. Hence, for making decision on N application rate, more useful information can be drawn from variables taken between 4 and 5 WAN. Overall, 224 kg N ha^{-1} produced significantly higher cane tonnage than the control plots at harvest. No interaction was found between variety and N rate on biomass yield and N uptake except 2014 first ratoon. Higher N application rate produced higher amount of inorganic N in surface soil which partly explained the higher yield obtained from these plots than the control plots. In case of first ratoon, the effect of N application rate was more significant than plant canes. The outcomes of this study also validate the concept of early-season crop sensing to estimate both yield and response to N application at harvest.

Billet-planted cane produced better stalk population and early-season plant population than whole stalk planted cane under congenial weather condition. In 2014, due to severe cold damage, there was no difference in stalk and plant population between these two planting methods. Poor germination and tiller production of billet-planted cane under adverse weather conditions was partly attributed to its low food reserve. Additionally, higher exposed surface in billets resulted in higher stem rot damage than the whole stalks. Early-season population stand was better in billet-panted cane than whole stalk-planted cane under favorable condition. At early tillering stage, CV among VI and plant population attained the highest r^2 value. Overall, among all VI, CV of $NDVI_{red}$ performed best. There was no effect of variety on canopy spectral reflectance. Planting material had no effect on yield except 2014 first ratoon. Sugarcane varieties produced significantly higher cane tonnage and sucrose than energy cane varieties, while energy

cane varieties produced significantly higher fiber. Among energy cane varieties, Ho 02-113 performed best in terms of cane yield and sucrose production (numerically). Only 2014 first ratoon showed interaction effect between variety and planting materials for fiber content.

The outcome of this research suggested that the sensors produced $NDVI_{red}$ and SR_{red} can still be used to estimate biomass yield of energy cane at early-season growth stage. Four WAN is the most sensitive stage to monitor N health status of energy cane and potentially, make decision on N application rate as it has similar N response pattern as harvest. Early tillering stage is an appropriate time to monitor cane population using CV among NDVI readings. This study also suggested that decision on selecting planting materials should be done based on the weather conditions of an area. Whole stalk-planted cane in areas under temperate region with high potential frost damage in winter will likely succeed than billet planted-cane. The success of incorporating CV to sensor-based N recommendation will enable variable N rate technology in cane to selectively dispense N fertilizer where it is needed in the field.

Vita

Saoli Chanda, a native to West Bengal, India received her Master's degree in Agronomy from Bidhan Chandra Krishi Viswavidyalaya, India in 2010. Thereafter she worked as a research assistant in principal agricultural office at Malda, West Bengal, India. To fulfill her aspiration of working on agronomic crop production, she joined graduate program of Louisiana State University in the School of Plant, Environmental, and Soil Sciences during spring 2012. She worked with Dr. Brenda Tubana on nitrogen management in energy cane production using remote sensing technology. Her dissertation title is "The agronomic use and application of canopy reflectance within the visible and near-infrared wavebands and its relation with nitrogen fertilization in energy cane production in Louisiana".