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Review

Enhancing agricultural research within West Africa using sensor-based technologies

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The global population of the world is on an exponential increase, expected to surpass 9 billion by the year 2050. This places a huge demand on food securities and the need to address the challenges facing food security. Sensor-based techniques [e.g., Fourier-transform infrared spectroscopy] have enhanced the understanding and diagnosis of several disease conditions, including cancers and are increasingly being applied to answer research questions in other areas including agriculture. Methods employed are relatively non-destructive, rendering samples reusable to be analyzed by more conventional approaches as well as allow the fingerprinting of biological samples based on the vibrational modes of the molecules within the sample. Spectra are derived consisting of wavenumber-absorbance intensities within a typical biological experiment and a complex dataset is quickly generated. Biological samples ranging from biofluids to cytology to tissue sections derived from human or sentinel organism sources including plants can easily be observed using this technique. Using a reference range of a designated normal state, anything lying outside this is judged as potentially atypical. Discriminating chemical entities can be identified using computational approaches, which allow one to minimize within-category confounding factors. Technologies involving sensor-based approaches provide a sensitive, cost effective technique for biological and agricultural research. Sensor-based techniques allow the characterization of biological material based on its biochemical-cell fingerprint and could enhance the study of plant species in agricultural research.

Key words: Biochemical-cell fingerprint, biospectroscopy, computational analysis, Fourier-transform infrared, infrared spectra, fluted pumpkin, *Telfairia occidentalis*.

INTRODUCTION

Technologies incorporating sensor-based techniques (SBTs) have been around for many years. They have more recently gained attention due to their applications to understand occurrences in biological and environmental science such as cancer aetiology, biomonitoring and more recently, Butler et al. (2017) document a possible method of observing specific nutrient deficiencies in plant using SBT. The sensitivity and resolution of most SBTs as well as the rapid generation of datasets representative of samples including the least variance detectable between samples make SBTs quite desirable.

Sensor-based techniques [e.g., Fourier-transform infrared (FTIR) spectroscopy, Raman spectroscopy] have been applied to understand several biological and

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environmental phenomena (Llabjani et al., 2011, 2014, 2012; Obinaju et al., 2014; Patel et al., 2011; Patel and Martin, 2010; Walsh et al., 2008, 2007b) and have significantly enhanced the study of molecular (bio-) chemistry of organisms including plants (Ivanova and Singh, 2003). They have been shown to differentiate between normal and diseased tissue types (Gajjar et al., 2013) as well as between nucleus and cytoplasm of cells (Holton et al., 2011). The possibility of studying a wide variety of sample types (Baker et al., 2014) using SBTs, is a significant advantage.

Environmental change is greatly modified by pollution and contamination, increased as a result of human and industrial activities (Callender and Rice, 1999). Increased contaminant concentrations have been shown in many urban and industrialized environments (Li et al., 2001; Zhang et al., 2005). Satterthwaite (1993) documents Waller (1991) as having identified urban air contaminants to include smoke/suspended particulates, sulphur dioxide, sulphuric acid, polycyclic aromatic hydrocarbons (PAHs) nitric oxide, carbon monoxide and some heavy metals. According to Obinaju and Martin (2013), a variety of these listed contaminants are found to be present in soil (Morillo et al., 2007) and water with increased contaminant concentration in most industrial areas (Li et al., 2001; Zhang et al., 2005). There is a vast amount of research on the effects of human exposure to environmental contaminants (Afroz et al., 2003; Brunekreef and Holgate, 2002; Dockery and Pope, 1994; Lin et al., 2007) and an equal amount of research on the effects of environmental contamination on agriculture, *e.g.*, plant disease and plant growth (Das et al., 1997; Heagle et al., 1973). The effects of environmental contamination and climate change are huge threats to agriculture and crop production e.g. nutrient deficiencies and low yield. This in turn impacts hugely on food security.

Understanding change in biological molecules

Disease processes are most commonly identified using biochemical pathways within organisms and the evaluation of the unique chemical “fingerprints” (metabolite formation or gene/protein alterations) that specific cellular processes leave behind (Kenny et al., 2005; Mollerup et al., 1999; Welthagen et al., 2005). These fingerprints are considered cellular biomarkers in biological cells and tissue samples, and may indicate exposure to factors capable of initiating or altering cellular processes (Obinaju, 2012; Obinaju and Martin, 2013). Analytical techniques such as chromatography, microscopy or spectroscopy/spectrometry are able to detect these biomarkers within cells or tissue samples (Bi et al., 2007; Zhang et al., 2004), using them as indications of biochemical toxicity, initiation and progression of disease conditions or the presence of certain chemicals within given samples.

SBTs, particularly infrared (IR) spectroscopy, generate a chemical signature based on the structure and function of different biological systems (Martin et al., 2010). This technique may be applied to study conformation in biomolecules because various chemical bonds absorb light at different wavelengths within the mid-IR region, generating a vibrational spectrum consisting of wavenumber-absorbance intensities, referred to as a “biochemical-cell fingerprint” (Walsh et al., 2007a). These wavenumber-absorbance intensities (peaks) are representative of the biochemical composition of the sample interrogated, where peak areas can indicate the concentration of the molecule (Cakmak et al., 2006; Obinaju et al., 2014; Severcan et al., 2005) and the position of peak centroids, a measure of structural integrity within the sample (Palaniappan and Pramod, 2011).

Techniques incorporating sensors undergo constant protocol modifications to increase precision, minimize/ eliminate bias, achieve certain sensitivity/selectivity levels and expand resolution. These modifications are mostly made possible by the application and use. Thus, this review focuses on the prospects of the application of SBTs particularly IR spectroscopy to enhance agricultural research by understanding the various changes occurring within plant samples as a result of interaction with environmental contaminants, including observing nutrient deficiencies in real-time.

PRINCIPLES AND METHODS

The interrogation of biological specimens using spectrochemical methods of analysis with the aim of acquiring information pertaining to an analyte of interest based on its inherent ability to absorb, reflect, bend, or scatter radiation as a consequence of its chemical bond structure could be defined as biospectroscopy and this method involves focusing the IR beam on a sample (cells or tissue sections), which absorbs the energy detected using methods which measure transmission, reflection or reflectance (Obinaju and Martin, 2013).

The analytical value of spectroscopy including IR spectroscopy is based on spectral bands occurring at more or less localized positions in the spectrum, which can be correlated to the presence/absence of characteristic structural features of the sample under study (Stuart, 2000). Plant cells or tissue samples could be interrogated using SBTs combined with suitable computational data processing approaches which allow the detection and measurement of biomarkers in the case of disease or a general response to environmental change (Obinaju et al., 2014). FTIR spectroscopy has been effectively been applied to study senescence in plant leaves (Ivanova and Singh, 2003).

It is generally understood that constituents of biological cells absorb in the mid-IR region ($\lambda = 2\text{-}20\ \mu m$) based on the chemical bonds present, with specific absorption regions and spectral bands attributed to lipids (≈1,750 cm^{-1}), carbohydrate (≈1,155 cm^{-1}), secondary structure of proteins (Amide I, ≈1,650 cm^{-1}; Amide II, ≈1,550 cm^{-1}), and DNA/RNA (≈1,225 cm^{-1}; ≈1,080 cm^{-1}) (Kelly et al., 2011). Using alterations in the spectral "biochemical-cell fingerprint" and peak assignments (Movasaghi et al., 2008) which typify the architectural structure of the cell or biomolecule under probe, the analyst can best understand mechanisms by which effects observed are possibly induced.

SAMPLE PREPARATION AND DATA HANDLING APPROACHES

IR spectroscopy, particularly attenuated total reflection FTIR (ATR-FTIR) spectroscopy techniques are applicable to a wide variety of sample types (Baker et al., 2014). However, each sample type requires adequate preparation prior to interrogation. For most spectroscopy, tissue cultures do not necessarily require elaborate preparation prior to interrogation aside from critical steps such as alcohol fixation and deposition on appropriate support matrix depending on technique (Obinaju and Martin, 2013). It is also important that sufficient amounts of material be deposited onto the support matrix to allow an absorbance reading of sufficient intensity (Martin et al., 2010). Generally, biospectroscopy techniques require the processing of the biological specimen with a view towards the capabilities of the technology to be employed (that is, a 10-μm-thick tissue section floated onto an IR-transparent substrate for transmission measurements) (Obinaju and Martin, 2013).

With excised tissue samples, considering the time lapse between excision and interrogation, it is critical to preserve tissue samples either by freezing in optimal cutting temperature (OCT) compound medium (Shim and Wilson, 1996) or embed tissues using paraffin wax in order to retain native biochemical states. The quality of spectral data obtained is dependent on sample handling and preparation techniques used prior to interrogation (Martin et al., 2010; Schwartz et al., 2003).

The interrogation of cells or tissue samples generates complex spectral data sets and necessitates the application of suitable data handling tools in order to extract important discriminating information (Obinaju and Martin, 2013) and several approaches exist for processing spectral data including peak picking (Garrett et al., 1991), regional integration of spectra (Chen et al., 2003), principal component analysis (PCA) (Jolliffe, 2005), linear discriminant analysis (LDA) (Martin, 2007) and machine learning algorithms, which allow for classification according to exposure and effect (Llabjani et al., 2012; Trevisan et al., 2012).

Interpretation of IR spectral datasets is based on a multivariate approach [PCA with or without LDA] (Martin et al., 2010) which allows the identification of wavenumber-related biomarkers of effect by reducing the initial number of variables present in each IR spectrum to a small number of factors. This approach has been extensively used in spectra data processing (Obinaju et al., 2014; Ukpebor et al., 2011; Walsh et al., 2008, 2007b).

TECHNIQUES AND APPLICATIONS OF SBT FOR AGRICULTURAL RESEARCH

These various SBTs, including FTIR spectroscopy, monitor the vibrational modes of functional groups within biomolecules and enable a correlation between chemical information and histological structures where shifts in peak positions, changes in bandwidth intensities and band area values of the IR bands are used to obtain valuable structural and functional information about the system of interest (Stuart, 2000). The sensitivity of IR spectroscopy has been greatly enhanced over time, enabling its application to answer various biological questions (Ahmad et al., 2008; Barber et al., 2006; Llabjani et al., 2012, 2010; Obinaju et al., 2014; Ukpebor et al., 2011).

Continuous environment change is most likely to impact agriculture as well as food security (Obinaju, 2010). The components of environmental change, that is, environmental contamination by chemical compounds, possess the potential to alter structural components as well as biochemical processes of plant and animal cells (Lewtas et al., 1993). These alterations particularly to plant cells could mean reduced growth/yield or the onset of disease in the affected plant species. Several biological techniques rely on the subjectivity of pathologists and simple statistical tests to determine or predict these effects and complex procedures/staining to enable section architectural visualization is adjudged time consuming, subjective and ultimately renders samples non-transferable to other techniques (Obinaju et al., 2014). However, this can be overcome using SBTs (Walsh et al., 2008). A major advantage of using SBT is that analyses can be conducted in the absence of any need for complex labelling or enzymatic requirements, as often needed by more conventional approaches (Obinaju and Martin, 2013).

Hypothetically, using the fluted pumpkin (*Telfairia occidentalis*) species as a potential case study with sample preparation and data acquisition processes as illustrated in Figure 1 and in Obinaju and Martin (2013), an example of the potential application of biospectroscopy in agricultural research is explained. Determining crop nutrient status particularly by foliar and soil analyses, relies currently on analytical techniques such as flame photometry and flame atomic absorption spectroscopy. These methods however efficient and sensitive, require

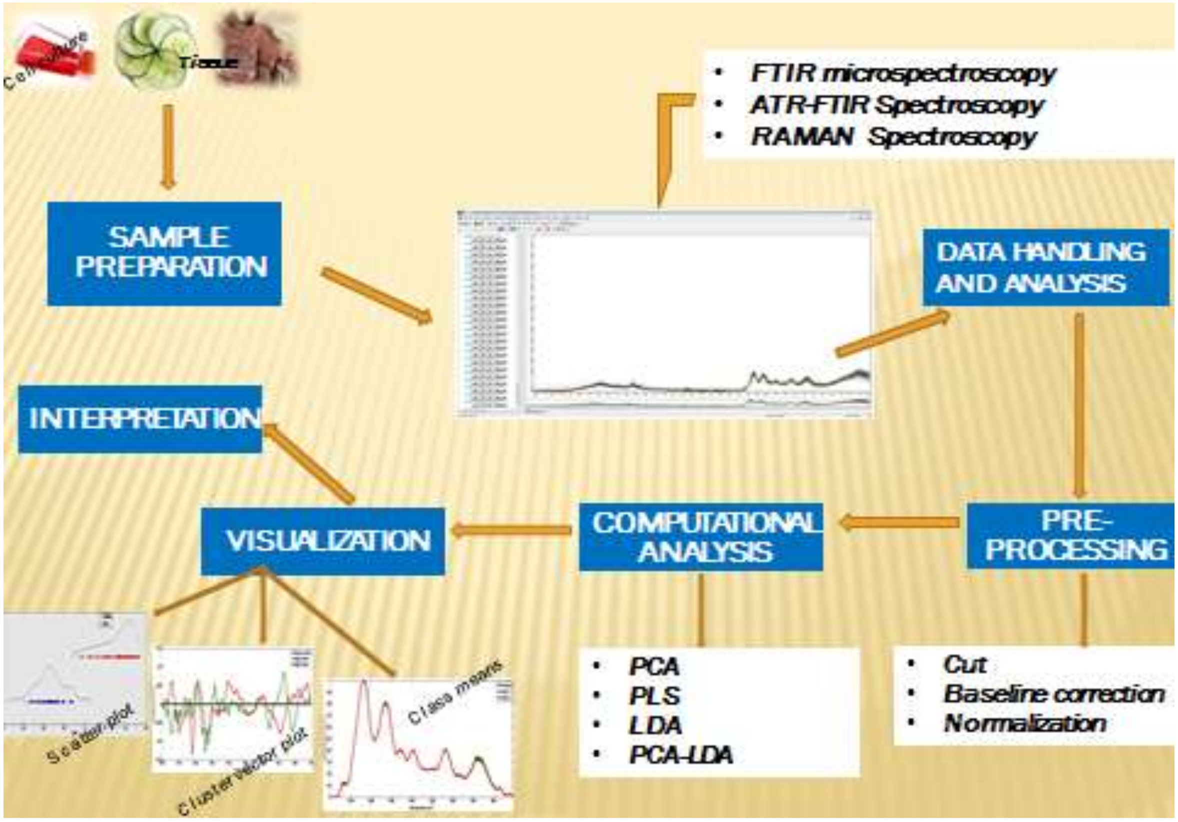

Figure 1. Schematic diagram showing the various processes involved in biospectroscopy analysis from sample preparation to data visualization and interpretation. Samples can either be (1) snap-frozen in liquid nitrogen or using optimal cutting temperature (OCT) compound medium; (2) embedded using paraffin wax; or (3) fixed in a mixture of alcohol and distilled water. Data interpretation can be done based on identified peaks and wavenumbers in plots/graphs.

a nutrient extraction step usually involving acid digestion which can often be time-limiting and may remove any information regarding spatial origin and distribution (Butler et al., 2017).

The increased awareness of the health protecting and promoting properties of consuming green vegetables has increased over the years. With increasing environmental change including environmental contamination, there is growing concern as to the possibility of exposure to contaminants *via* dietary consumption and the effects of soil contamination on the growth parameters of dietary crops especially fruits and vegetables.

T. occidentalis is a tropical vine and a member of the Curcurbitaceae family grown in West Africa as a leaf vegetable, for its edible seeds and more importantly its rich nutritional and perhaps therapeutic value (Egba et al., 2014). There are genetic variants of the fluted pumpkin species particularly within Nigerian states (Fayeun et al., 2012) with certain levels of variation in their individual vegetative characteristics, *e.g.*, vine length, foliage width and number of branches per plant (Aremu and Adewale, 2012; Cyril et al., 2014; Fayeun et al., 2012). The distinction between each variant could easily and more rapidly be detected based on the mean spectra and cluster vector plots derived using IR spectroscopy coupled with multivariate computational analysis. The various intensities and peak areas could provide a measure of the concentration of each desirable molecule/nutrient within the parts of the interrogated plant. Furthermore, in cases where the interrogated plant is exposed to an element of environmental change, that is, chemical contaminant or UV radiation, a comparison of the spectra of exposed plant, to the spectra of a control or unexposed plant could provide an indication of the possible areas of the plant targeted by the said element, *e.g.*, decreased absorption bands for cellulose (1030 cm^{-1}) indicating changes to leaf pigmentation (Obinaju et al., 2014), changes to the polysaccharide (Pectin 1055 cm) region as an indication of calcium (Ca) deficiency in plant tissue (Butler et al., 2017).

Conclusions

Food security is a major threat and requires novel and innovative, not to mention rapid yet cost effective methods of dealing with the challenge, especially through agricultural research. SBTs offer improved understanding and characterization of molecular alterations in both plant and animal species especially those occurring as a result of exposure to environmental contaminants (Ahmad et al., 2008; Obinaju et al., 2014; Ukpebor et al., 2011) because methods employed are specific and sensitive to changes within the biochemical constituents of cells and tissues at certain wavelengths (Baker et al., 2014) and while spectral analysis may require basic knowledge of statistical software packages such as MATLAB®, the various techniques provide a suitable avenue to sort for desirable traits, monitor plant nutrient stress in real-time before the manifestation of symptoms indicating deficiency and track the activity of environmental contaminants and pathogens in agricultural species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Chemical attributes of the soil and agronomic characteristics of maize as a monocrop and intercropped with herbaceous and woody legumes in the savannah of Roraima, Brazil

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Several studies seek to identify management systems that promote an increase in soil quality. As such, the aim of this study was to evaluate the effect of combinations of herbaceous and woody legumes on the productivity of green maize and the chemical attributes of the soil. The research was developed over two crop years (2016 and 2017). The experimental design was of randomized complete blocks with three replications and subdivided plots, in a 2 × 5 factorial scheme, representing the presence and absence of an alley cropping system with gliricidia, and five intercrops of maize with legumes: Maize + jack bean planted at the same time; maize + pigeon pea planted at the same time; maize + velvet bean planted 15 days after the maize; maize + *Crotalaria juncea* planted at the same time; single maize crop. The alley system increases the total and commercial productivity of husked cobs in maize intercropped with velvet bean. Intercropping maize with legumes under an alley system helps to increase the levels of phosphorus and potassium in the soil. Irrespective of the type of intercrop, green manure with legumes increases the soil organic matter content of farmed land in Roraima.

Key words: Alleys, gliricídia, agroforestry system, green manure.

INTRODUCTION

Disordered use and occupation of the land has caused several environmental changes that are often irreversible. Magalhães et al. (2013) found that substituting the forest for another type of land use can lead to significant losses in organic matter and other soil nutrients, changing its dynamics, and consequently altering the input and output of nutrients in the system (Durigan et al., 2017).

To minimise the damaging effects of conventional farming on the environment, studies have been developed to evaluate management systems that maintain or

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improve soil quality (Salmi et al., 2009; Oliveira and Bueno, 2016; Xu et al., 2019). As such, agroecological farming is emerging as one way of relieving environmental problems, following the principle of a rational use of natural resources. The use of legumes can be an alternative for recovering and improving the soils of degraded areas, since legumes use their own vegetation to protect the soil, in addition to the large deposition of plant biomass on the ground that contributes to improve the chemical and biological attributes of the soil through incorporation (Nogueira et al., 2012; Abreu et al., 2016; Oliveira et al., 2016). Soil conditions can also be improved by the use of species of woody and herbaceous legumes that are capable of associating with atmospheric nitrogen-fixing bacteria and with mycorrhizal fungi (Habiyaremye et al., 2018). This technique is considered low cost and can give promising results (Nogueira et al., 2012).

Under an alley cropping system, annual plants are grown between rows of woody legumes that are periodically pruned and their branches deposited on the crop rows, thereby transferring nutrients from the trees to the agricultural crops. Decomposition of the branches of the legume species in the soil increases the organic matter content, the availability of nutrients, the cation exchange capacity and the production of organic acids (Moura et al., 2012; Santos et al., 2012; Aguiar et al., 2013). In this way, green fertilisation of the maize from the intercrop with legumes can promote increased productivity in the crop of commercial interest, and at the same time, the production of plant biomass in the species that make up the intercrop can improve the chemical attributes of the soil, with positive effects on crop development (Castro and Prezzoto, 2008; Fanish, 2017).

In view of the above, the aim of this study was to evaluate the effect of combinations of woody and herbaceous legumes on the productivity of green maize and on the chemical attributes of the soil.

MATERIALS AND METHODS

The work was carried out from April 2016 to November 2017, and consisted of an experiment under rainfed conditions, conducted in the experimental area of the Sector for Olericulture of the School of Agricultural Technology, on the Murupu campus of UFRR, 37 km from the town of Boa Vista in the state of Roraima, 07° 28' 14” S and 34° 48' 31” W.

According to the Köppen classification, the climate in the area is type Aw, characterised as tropical rainy, hot and humid, with a distinct dry period following alternating periods of wet and dry. The mean temperature is around 25°C, and the annual rainfall in the study area was around 1,600 mm, with irregular distribution over two distinct wet periods and the most rainfall between April and September. The mean annual relative humidity ranges from 70 to 80% (Alvarez et al., 2013).

The experiment was conducted on two strips of farmed land, under an agroforestry system of alley cropping planted in April 2013. The chemical attributes of the soil are shown in Table 1. After the results of the soil analysis, soil preparation began in May 2013 with liming, applying 2.0 t ha^{-1}, followed by a light harrowing to incorporate the existing vegetation. The gliricidia was planted using cuttings from the Experimental Unit of EMBRAPA Roraima in the District of Mucajaí. Alleys of gliricidia were planted at a spacing of 3.5 m between rows and 2.5 m between plants. After planting the cuttings, the seeds of the herbaceous legumes (crotalaria, pigeon pea, jack bean and velvet bean) were sown, following the distribution of the experimental design. Pruning management of the gliricidia plants began eight months after planting the cuttings, when the stems were over 1.5 m in height; any leaves and branches smaller than 1.5 cm in diameter were then spread over the ground.

After planting the alleys and sowing the legumes, the soil in the experimental area remained fallow until May 2015. Maize intercropped with herbaceous legumes (crotalaria, pigeon pea, jack bean and velvet bean) was then grown over two consecutive years. Activities for planting the system started with soil samples, collected from areas with and without gliricidia. Ten single samples were collected from each area at a depth of 0-20 cm to form one composite sample as illustrated in Table 2.

The experimental design was of randomised complete blocks, with three replications and subdivided plots, in a 2 × 5 factorial scheme, representing the presence and absence of an alley cropping system in the main plot, and five intercrops of maize with legumes in the subplots C1 = maize + jack bean planted at the same time; C2 = maize + pigeon pea planted at the same time; C3 = maize + velvet bean planted 15 days after the maize; C4 = maize + *Crotalaria juncea* planted at the same time; C5 = control (single maize crop), with three replications.

Before sowing the maize and legumes, the gliricidia plants in each area were pruned to a height of 2.0 m. The experiment began in May 2016 by planting the crops (creole maize and shrub-like legume species). The cover species and maize were sown manually in furrows, at a spacing of 0.70 × 0.25 m for both the intercropped maize and the single maize crop. Each subplot consisted of nine rows of 10 m, four rows of maize and five rows of legumes inserted between the rows of maize.

The seeds were distributed in the furrows: Four seeds per linear metre for the maize and three seeds per linear metre for the cover species such as velvet bean and jack bean; for the pigeon pea and *Crotalaria juncea*, 20 seeds were used per linear metre. After the plants had emerged, thinning was carried out to adjust the desired population per hectare.

Weeding and heaping were carried out during the experiment, in order to keep the area free of spontaneous plants and stimulate the development of secondary roots in the maize. It is important to point out that 17 days after planting the maize, the area with no gliricidia needed weeding, with four weedings carried out during each crop. In the area with gliricidia, three weedings were carried out during the first crop and two during the second, the first weeding taking place after 25 and 30 days respectively due to the incidence of spontaneous plants. To avoid competition for light, the gliricidia plants were again pruned 40 days after each planting. The pruned branches were deposited between the crop rows in the area with gliricidia. Every 15 days, preventive applications of neem extract (*Azadirachta indica*) were carried out to avoid the incidence of insect pests in the cultivated area. In order to help maize development, cover fertilisation was carried for with each crop, with 80 kg ha^{-1} N in two applications, 20 and 45 days after sowing the maize and legumes. Urea (45% N) was used as the source of N. It is important to note that only the agronomic characteristics of the maize from the 2017 harvest were determined; the maize grown in 2016 was harvested but its agronomic characteristics not verified, with all the straw produced remaining in the experimental area. Only the chemical characteristics of the soil were determined for both 2016 and 2017.

The green maize was harvested manually once the cobs were physiologically mature, when the grains presented a yellow, milky endosperm with a water content of 70% to 80%. At the time of harvesting, the height of the plants and of the first ear insertion

Table 1. Chemical attributes of the soil in the experimental area at a depth of 0 to 20 cm.

pH	P	K	Ca	Mg	Al	H+Al	CEC	SB	OM	V
	(mg dm^{-3})	cmol$_c$ dm^{-3}							%	
4.8	4.6	0.06	0.5	0.2	0.23	2.0	5.0	0.75	0.7	27

pH in water = ratio1.0:2.5; P = Mehlich 1 extraction; CEC = cation exchange capacity; SB = sum of bases; OM = organic matter; V = base saturation.
Source: Laboratory: LABRAS, Minas Gerais, 2013.

Table 2. Chemical attributes of the soil in the experimental area at a depth of 0 to 20 cm on the land with and without alleys of gliricidia.

System of alley	pH H_2O	P	K	Ca	Mg	Al	H + Al	OM
		mg dm^{-3}	cmol$_c$ dm^{-3}					(dag kg^{-1})
With	5.7	15.9	0.10	1.0	0.4	0.06	1.5	1.1
Without	5.6	10.3	0.13	0.9	0.3	0.09	1.4	0.9

pH in water = ratio1.0:2.5; P = Mehlich 1 Extraction; OM = organic matter.
Source: Laboratory: LABRAS, Minas Gerais, 2013.

were determined using a metric tape in ten representative plants from each subplot; plant height was measured from ground level to the insertion point of the final leaf and the height of the first ear insertion was determined from ground level to the base of the ear. After harvesting, all the cobs from the working area of each subplot were husked and weighed on a balance for later estimation of productivity. After removing the husks, the length of the cobs was determined using a ruler graduated in millimetres. A 150 mm digital calliper was used to determine the diameter. The commercial productivity of the husked cobs was based on the commercial fresh weight of the cobs obtained in each subplot, with the results expressed in kg ha^{-1}. As per local market demand, a commercial cob was considered to have a length greater than 16 cm and a diameter greater than 4 cm, with no defects, such as a lack of seeds or damage caused by pests. At the same time, the total productivity of the husked cobs was determined from the total fresh weight of the cobs obtained in the working area of each subplot, with the results expressed in kg ha^{-1}.

For the chemical analysis of the soil in the experimental area, soil samples were collected at three stages of the experiment. The first collection was made before the experiment was set up in the areas with and without gliricidia. The second and third samples were taken two months after each maize harvest when four composite samples were collected each time, consisting of 10 single samples for each crop, as follows: Sample 1 = collected in the subplot of maize intercropped with legumes in the presence of gliricidia; sample 2 = collected in the subplots of single maize crop in the presence of gliricidia; sample 3 = collected in the subplots of maize intercropped with legumes in the absence of glyricidia; and sample 4 = collected in the subplots of single maize crop in the absence of gliricidia. After collection, the samples were air dried, sieved through a 2 mm mesh and sent to the LABRAS Laboratory for evaluation of the chemical characteristics of the soil in the experimental area. The analysis followed a methodology proposed by EMBRAPA (2011).

The pH was determined in water, measuring the effective concentration of H^+ ions in the soil solution by means of a combination electrode immersed in a soil suspension of water in the ratio of 1:2.5. To determine the phosphorus content, the Mehlich 1 extracting solution, also known as the double-acid or North Carolina solution, was used; the extracted phosphorus was determined spectroscopically by reading the colour intensity of the phosphomolybdic complex. Potassium was determined by extraction with dilute hydrochloric acid solution followed by flame spectrophotometry. Organic matter was determined by the colorimetric method, using sulphuric acid and sodium dichromate.

The data were submitted to analysis of variance and the mean values of the treatments compared by Scott-Knott test at 5% probability. The SISVAR statistical software (Ferreira, 2014) was used in analysing the data.

RESULTS AND DISCUSSION

According to the summaries of the analysis of variance, it was found that all the variables under evaluation relative to the agronomic aspects of the green maize showed significant interaction between the treatments being studied, except for plant height (PH) and ear insertion height (EIH) as described in Table 3.

Among the variables under evaluation in the maize crop, plant height and ear insertion height were only influenced by the intercrop of maize and legumes; from the results, it can be seen that the intercropped maize showed greater values for plant height as shown in Table 4. This behaviour may have occurred due to the greater N demand of the maize during the early stages of its development, since all the plant material from the previous crop had already decomposed, and its nutrients released into the soil, resulting in greater nutrient availability for the plants. The greatest heights for ear insertion were recorded in the treatments of maize intercropped with crotalaria and jack bean, and in the single maize crop as presented in Table 4.

Castro and Prezzoto (2008) evaluated the performance of maize in a green manure system, verified that the

Table 3. Summary of the analysis of variance by mean square, relative to plant height (PH), ear insertion height (EIH), cob length (CL), cob diameter (CD), total productivity (TP) and commercial cob productivity (CCP) in green maize produced under an alley system and intercropped with herbaceous legumes.

SV	DF	Mean square				TP	CCP
		PH	EIH	CL	CD		
Block	2	409.80^{ns}	199.81^{ns}	0.04^{ns}	0.72^{ns}	1310745.03^{ns}	345721.28^{ns}
Alley (A)	1	240.83^{ns}	116.42^{ns}	0.13^{ns}	0.36^{ns}	101920.06^{ns}	80082.44^{ns}
Residual a	2	227.26	25.28	1.84	0.28	2928510.94	1670733.7
Intercrop (I)	4	13116.33*	421.07*	10.90**	2.74*	13841889.37**	12043686.98**
A x I	4	1457.08^{ns}	175.65^{ns}	3.20**	11.43**	6096943.95*	12990620.51**
Residual b	16	412.-5	90.16	0.61	0.85	1311877.45	600025.36
Total	29	-	-	-	-	-	-
CV a (%)	-	4.57	3.37	8.68	1.33	23.1	27.14
CV b (%)	-	8.69	6.36	5.02	2.29	15.46	16.27

SV = Source of variation; DF = Degree of freedom; ns, *, ** = Not significant, significant at 5% and significant at 1% probability respectively by F-test; CVa = coefficient of variation of the main plot; CVb = coefficient of variation of the subplot.

Table 4. Mean values for plant height and height of the first ear insertion in cm, as a function of intercropping with crotalária juncea (CJ), pigeon pea (PP), jack bean (JB), velvet bean (VB) and single maize crop (SM).

Intercrop	Plant height	Height of ear insertion
Maize + CJ	241.33^{a}	151.1^{a}
Maize + PP	245.50^{a}	145.8^{b}
Maize + JB	253.00^{a}	156.7^{a}
Maize + VB	234.16^{a}	136.8^{b}
Single maize	193.50^{b}	156.6^{a}

Mean values followed by the same letter in the columns do not differ from each other by Scott-Knott test at 5% probability.

height of the plants was higher using the consortium with the pork bean, but the authors affirm that there is no difference between the height of the spikes among treatments without green manure, and intercropped with grass, pork beans, guandú beans and white mucuna. Gitti et al. (2012) also verified that the simultaneous intercropping of corn with *Crotalaria juncea* and *Crotalaria spectabilis* did not affect plant height, and it can be deduced that there was no competition for light between maize and legumes. In the present research, the results indicate the absence of interspecific competition and the benefits of legume intercrops for maize growth.

Under the alley system, the greatest cob diameter was recorded for maize intercropped with velvet bean (4.32 cm). There was no statistical difference between the other intercrops as shown in Table 5. When grown with no alleys, the maize cobs showed the greatest diameter for the intercrop with jack bean (4.26 cm). Castro et al. (2014), working with different cover crops and the P4285H hybrid maize cultivar in Araxá, Minas Gerais (MG), found higher values than found in this study, with the crotalaria affording an average diameter of 5.47 cm, greater than the control (5.12 cm) and equal to the other treatments.

Under the agroforestry system, the greatest cob length was seen in the intercrop of maize and jack bean, with an average length of 17.1 cm. In the area with no gliricidia, the greatest cob length was also seen in the intercrop of maize and jack bean, with a mean length of 18.5 cm as described in Table 5. This superiority of the jack bean compared to the other intercrops may be related to its growth habit, since the jack bean does not compete with the maize for light. These results agree with those of Santos et al. (2010), who recorded greater lengths for maize cobs when grown intercropped with jack bean. However, Torres et al. (2014) working with cover crops and nitrogen fertiliser in a crop of off-season maize, recorded no statistical difference for the length or diameter of the cobs. Growing maize under an alley cropping system, the total productivity of husked cobs showed no difference between the intercrops, while for commercial cob productivity, the treatments that provided the greatest weight were the intercrop with crotalaria (5,600.04 kg ha^{-1}) and with jack bean (5,299.42 kg ha^{-1}), there being no statistical difference between the two as shown in Table 6. However, the single maize crop without

Table 5. Mean values for diameter and length (cm) in maize, as a function of intercropping with *Crotalaria juncea* (CJ), pigeon pea (PP), jack bean (JB), velvet bean (VB) and in the single maize crop (SM), with and without alley cropping.

Alley		Crotalaria	Jack bean	Pigeon pea	Velvet bean	Single maize
Ear diameter (mm)	With	3.94Ab	4.09Ab	3.97Bb	4.32Aa	4.01Ab
	Without	3.94Bc	4.05Ab	4.26Aa	3.86Bc	4.11Ab
Ear length (cm)	With	15.7Ab	15.6Ab	17.1Ba	14.9Ab	14.8Bb
	Without	14.1Ac	16.2Ab	18.5Aa	13.6Ac	16.3Ab

Mean values followed by the same uppercase letter in the columns and lowercase letter on the rows do not differ by Skott-Knott test at 5% probability.

Table 6. Mean total productivity and commercial productivity of husked maize cobs (kg ha^{-1}), as a function of intercropping with *Crotalaria juncea*, pigeon pea, jack bean, velvet bean and in the single maize crop, with and without alley cropping.

Alley		Crotalaria	Pigeon pea	Jack bean	Velvet Bean	Single Maize
Total husked cob productivity (kg ha^{-1})	With	8.788.4Aa	8.098.0Aa	7.126.3Aa	7.353.6Aa	7.421.0Ba
	Without	6.221.8Ab	6.957.0Ab	8.607.0Aa	3.806.2Bc	9.259.6Aa
Commercial cob productivity (kg ha^{-1})	With	5.600.04Aa	4.285.37Ab	5.299.42Ba	4.062.00Ab	3.279.37Bb
	Without	4.357.91Ab	3.978.51Ab	7.412.44Aa	1.333.00Bc	8.013.58Aa

Mean values followed by the same uppercase letter in the columns and lowercase letter on the rows do not differ by Skott-Knott test at 5% probability.

Table 7. Mean values for pH, phosphorus (P) and potassium (K) in the 0-20 cm soil layer, in the first (2016) and second (2017) crop of maize intercropped with herbaceous legumes and alleys (M+L+A), of maize and alleys (M+A), of maize intercropped with herbaceous legumes (M+L) and of a single maize crop (SM).

Treatment	1st year (2016)			2nd year (2017)		
	pH	P	K	pH	P	K
		mg dm^{-3}			mg dm^{-3}	
M+L+A	5.6	9.4	25	5.6	15.9	36
M+A	5.1	6.6	21	5.7	9.4	33
M+L	5.3	10.5	23	5.7	14.7	40
M	5.8	7.1	23	6.4	16.5	42

Treatments: M+L+A = maize intercropped with legumes in alleys; M+A = single maize crop in alleys; M+L = maize intercropped with legumes with no alleys; and, M = single maize crop.

alleys favoured greater total (9,259.6 kg ha^{-1}) and commercial productivity (8,013.58 kg ha^{-1}) of husked cobs, showing no difference to the intercrop with jack bean. These results show that despite results close to those obtained for the single crop, there may have been competition between the legumes and the maize.

For Aguiar et al. (2010), the cultivation in a system of alleys and with green fertilization in soils of the pre-Amazon region contributes to increase crop production due to physical-chemical improvement of the soil. In the present research, the higher productivity in the cultivation of single maize may be related to plant population. The trend of the results was also verified by Madalon et al. (2016), testing different spacing in an intercrop of jack bean and maize, in which they found the intercrop influenced a reduction in maize productivity compared to maize conventionally fertilised with NPK. In relation to the chemical characteristics of the soil, each combination of legumes, maize and alleys contributed to increase soil fertility during both crops as shown in Table 7.

The pH of the soil before setting up the experiment was 4.8, 5.6 and 5.7 in the farmed area, the area with no gliricidia, and the area with gliricidia respectively as described in Tables 1 and 2. Each of the values obtained for pH after the first and second year of cultivation were therefore higher than in the farmed area as shown in Table 1; however, the area of single maize crop with no gliricidia showed a greater increase in pH, and was

Table 8. Soil organic matter (OM) in the 0-20 cm layer during the first (2016) and second (2017) years of maize intercropped with herbaceous legumes and alleys (M+L+ A), of maize and alleys (M+A), of maize intercropped with herbaceous legumes (M+L) and of a single maize crop (SM).

Treatment	1st year (2016)	2nd year (2017)
	OM (dag kg^{-1})	
M+L+A	1.3	1.8
M+A	1.1	1.0
M+L	1.2	1.1
M	1.2	1.1

Treatments: M+L+A = maize intercropped with legumes in alleys; M+A = single maize crop in alleys; M+L = maize intercropped with legumes with no alleys; and, M = single maize crop.

higher than verified before planting. Furthermore, it can be seen that in the areas of intercropping (M+L+A) there was a small reduction in pH during the first (2016) and second (2017) year of cultivation, compared to values prior to the first year as presented in Table 2. As such, it was found that green manure with herbaceous and woody legumes were not enough to increase soil pH, but kept the acidity under control.

Aguiar et al. (2013) also saw higher values for pH under an alley cropping system. However, Barreto et al. (2010), testing areas under a system of alleys with gliricidia, single maize and secondary forest, at sampling depths of 0-5, 5-10, 10-20 and 20-40 cm, did not find any differences between the areas under evaluation. The phosphorus content of the soil increased under each treatment at the end of two years cultivation as presented in Table 7 in relation to the initial soil analysis before planting the maize as described in Table 2; however, during the first year there was a reduction in P levels under almost all treatments, except for the area of maize intercropped with legumes with no alleys (M+L). The levels of this element at the end of the first year of cultivation were 9.4, 6.6, 10.5 and 7.1 mg dm^{-3}, and 15.9, 9.4, 14, and 16.5 mg dm^{-3} at the end of the second year, under the M+L+A, M+A, M+L and M treatments respectively as shown in Table 2. The results show that the intercrop of maize with herbaceous legumes has a positive influence on P levels in the soil, and results in a greater capacity for releasing phosphorus adsorbed from the soil. The values for P obtained at the end of the second year were reflected in the productive performance of the maize, increasing productivity in areas of single maize and in areas with alleys of gliricidia, and although the greatest increase in P was seen in areas with no alleys, in the intercropped area with alleys (M+L+A), P remained at the same value seen at the beginning of the first year of cultivation, even with the nutrient exported for maize production and assimilated by the legumes. According to Aguiar et al. (2013), no-tillage under alley cropping increases the phosphorus content of the soil. It should also be emphasised that the levels of phosphorus influenced cob productivity. As such, Silva et al. (2014) point out that the levels of nitrogen and phosphorus in the soil are essential for increasing maize productivity.

For the potassium content, as seen with the phosphorus content, there was a reduction in the levels of the nutrient during the first year of cultivation; this could have occurred due to the lack of K fertilisation, and a part of what was in the soil being assimilated by the maize and legumes. Despite the reduction in K levels in the area with alleys, the reduction was less than in the open area, which can be explained by assimilation of this nutrient in the biomass of the gliricidia. Santos et al. (2010) found that the K concentration in plots fertilised with gliricidia biomass was greater during the first year of maize cultivation than found in the control without any fertilisation, explaining the accumulation of this nutrient in the biomass of the legume. During the second year of cultivation, K levels were higher in relation to the first year of cultivation, possibly influenced by decomposition of the plant biomass increasing the levels of the element in the soil. Agreeing with this result, Santos et al. (2010) found no difference in potassium content during the second year of cultivation between the treatments under study.

When considering the initial organic matter content of the soil (0.7 dag kg^{-1}, as presented in Table 1), it can be seen that the greatest accumulation during two years of cultivation was under the M+L+A treatment as shown in Table 8. This is probably due to the greater contribution of plant residue at the soil surface as illustrated in Table 8. However, irrespective of treatment, the organic matter content of the soil recorded at the end of the first and second years of cultivation was greater than the value seen before setting up the experiment as seen in Table 1. Similar results were seen by Loss et al. (2010), who found that OM is affected by the type of management in the area, where a greater amount of straw on the surface of the soil promotes increases in the levels of OM. In this sense, the results demonstrate the importance of the system of cultivation in alleys and of the green manure in the construction of soil fertility in the northern region of Brazil, especially in the savannah areas of Roraima. Moreover, the results express the importance of the use of agroecological practices in the maize cultivation system.

Conclusion

Intercropping maize with jack bean increases the diameter and length of the maize cobs in areas with and without alley cropping. The alley system increases the total and commercial productivity of husked cobs in maize intercropped with velvet bean. Intercropping maize with legumes under an alley system helps to increase the levels of phosphorus and potassium in the soil. Irrespective of the type of intercrop, green manure with legumes increases the soil organic matter content of agricultural land in Roraima.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Efficiency of *Cajanus cajan* in different sowing densities on soil compacting

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This research conducted in the District of Concepción, Concepción Department, Paraguay, involved the use of a randomized complete block design consisting of four treatments and five repetitions. The general objective was to evaluate the effect of *Cajanus cajan* L. species with different planting densities on soil compaction and as specific objectives; measure the compaction of the soil at the beginning and end of the crop cycle, determine the soil moisture percentage as well as the root development of the species at the end of the crop cycle. The data obtained were subjected to analysis of variance (ANOVA) and the means compared through the Tukey test at 5%. Determinations of resistance to soil penetration before the crop establishment and at the end of the crop cycle were not influenced positively; except at 20 cm of density that showed difference at the end of the cycle, the soil moisture percentage was not significant and for root development highly significant differences were found. As regards the soil compaction, it was found that the biological material used with different planting densities had positive effects in the compaction of the soil. Thus, the recommended density is 114,284 plants per hectare.

Key words: Compaction, root development, soil moisture.

INTRODUCTION

Soil compaction is an important feature that affects many aspects of agricultural soils, and is an indicator of the degree of compaction. The compaction limits the root growth and amount of air and water available to the roots (Herrick and Jones, 2002).

The main causes of the degradation of soil structure are related to the climate regime, geomorphological conditions, the intrinsic characteristics of the soils, trampling of animals, and above all the conventional tillage practices, regardless of its being either the wheel forces of the machinery or agricultural implements, especially when the soil is wet or saturated, at which time the soil is more prone to deformation.

Penetrometers are used worldwide to determine the resistance to soil penetration expressed as force per unit area of the cross section of the cone base (Bengough et al., 2001).

The maintenance and recovery of physical

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characteristics can be made feasible by the adoption of soil management practices, especially where a system of crop rotation includes plant species with an aggressive and abundant root system along with high biomass production, contributing to diminish the effects of soil compaction (Cubilla et al., 2002).

The use of green manure helps to protect and recover compacted soil, contributing to the restoration of highly degraded soils through the contribution of organic matter. The species *Cajanus cajan* is one of the alternatives for the most degraded soils because it is a plant with the capacity to adapt to diverse soils and climates, of fast growth and high production power of vegetative material; its root system is deep and reaches up to 3 m, which allows it to survive in conditions of extreme drought. It is a plant with the capacity to fix a high amount of nitrogen in the soil, and root that allows decompaction of soils (Salisbury and Ross, 2012).

The cultivation of this legume offers advantages as soil decompactant. Its roots are deep, act as subsoiler of the soil and can reach up to more than five meters deep, breaking the hard layers (plow foot), which in most small farms are 15 to 20 cm deep (GTZ, 2007).

The general objective was to evaluate the effect of the *C. cajan* species with different densities on soil compaction, and as specific objectives; measure the compaction of the soil at the beginning and end of the crop cycle, determine the percentage of soil moisture as well as root development of the species at the end of the crop cycle.

MATERIALS AND METHODS

The research work was conducted in the town of Huguá Ocampo, 27 km from the city of Concepción, Department of Concepción - Paraguay, circumscribed to the geographic coordinates' 23° 20'35.5'' South latitude and 57° 11' 47.7'' West longitude along with 200 msnm. The climate of the region was classified as frankly humid with an average annual temperature of 12°C and an average annual precipitation of 1000 to 1200 mm. The soil belongs to the large group Alfisol and subgroup Mollic, with panorama in the shape of a sandstone hillock, flat relief of 0 to 3% slope, an approximate height of 200 m above sea level, good drainage and zero rock, plus a climate transition between a Mediterranean type (López et al., 1995).

The sampling for the chemical and physical characterization of soil was conducted before the beginning of the experiment, the results of which were; pH_{water} 6.77; O.M. 0.59%; Al_3meq/100 g soil 0.00; P mg/L 5.76; K cmol/L 0.19; Ca cmol/L 3.07; Mg cmol/L 0.60; S mg/L 7.13; Fe mg/L 74.27; Cu mg/L 2.78; Zn mg/L 3.76; Mn mg/L 181.72; cation exchange capacity cmol/L 6.01; Ca/K 16.19; Ca / Mg 5.12; Mg/K 3.17; and Textures 18% clayey (CETAPAR, 2015).

The experimental design was randomized complete blocks, with four treatments and five replicates. The experimental units were constituted by parcels of 6 × 5 m, constituting 30 m^2 (EU) experimental units. Also, different densities were used in each treatment of pigeonpea (*C. cajan*). The description of the treatments can be seen in Table 1.

Preparation begins with the cleaning and later the delineation of the experimental plot. Before the establishment of culture, the soil penetration resistance was measured using a cone penetrometer (analog) in each subplot and soil samples were taken to measure its moisture.

The *C. cajan* L. sowing was done by manual seeder at different densities after a good rainfall to guarantee the emergence of the seeds. At 30 days after sowing, the weeds were cleaned manually using a hoe.

The determinations of penetration resistance and soil moisture percentage were evaluated before sowing and at the end of the crop cycle at 210 days after sowing; thereafter, root development was measured at the end of the crop cycle.

Resistance to penetration

This was evaluated at depths of (10, 15 and 20 cm) obtaining 15 samples from each EU, with the use of the penetrometer, expressed in Mega pascal (MPa) (Reichert et al., 2003).

Percentage of soil moisture

The soil moisture percentage was measured for one sample per EU, totaling 20 (twenty) soil samples, by a shovel to a depth of 0.20 cm, and were thereafter introduced into containers with 500 g soil each measured by a precision scale and dried in a stove. After 72 h, the samples were weighed on a precision scale and the weight compared with the initial weight, resulting to the percentage of moisture present in the soil of each experimental unit (Reichert et al., 2003).

Root development

The roots of *C. cajan* were measured from the base of the plant neck to the tip of the root. For the measurements and evaluations referring to the distribution of the root system of the crop, the crop profile method described by Böhm (1979) was applied.

Data analysis

The evaluated data were subjected to analysis of variance (ANOVA) and the variables that presented significant statistical differences were compared using the Tukey test at 5% of probability with the statistical program ASSISTAT version 7.7 beta (De Sousa, 2013).

RESULTS AND DISCUSSION

Table 2 shows the results of the obtained means, where the variable of resistance to the penetration of the soil before planting and at the end of the crop cycle does not present a significant difference at the depths evaluated, except at the depth of 20 cm which had a positive effect at the end of the crop cycle.

We can verify that the treatments evaluated with the depth of 10 cm presents lower resistance of soil to the penetration compared with the other depths, whereas as the depth of the soil increases it shows that the soil resistance increases with 15 and 20 cm, to give 4.21 and 4.71 MPa, respectively.

The coefficient of variation before sowing was: at 10 cm, 6.55%; at 15 cm, 7.47%; and at 20 cm, 7.04%, and at the end of the crop cycle reaches 6.83% at 10 cm;

Table 1. Description of the treatments used.

Treatment	Description	Plants per hole	Density (plant ha^{-1})
T1	0.50 between holes cm × 1.00m between rows	3	60.000
T2	0.30 between holes cm × 0.80 m between rows 2 83.333	2	83.333
T3	0.25 between holes cm × 0.70 m between rows 2 114.284	2	114.284
T4	0.20 between holes cm × 0.50 m between rows 2 200,000	2	200.000

Source: Paraguay (2016).

Table 2. Variation of compaction level in the sowing densities of *Cajanus cajan*.

Depth (cm)	Treatment	Penetration resistance (MPa)	
		Before sowing	Crop final cycle
10	T1	3.36[a]	1.73[a]
	T2	3.30[a]	1.68[a]
	T3	3.39[a]	1.69[a]
	T4	3.36[a]	1.73[a]
15	T1	3.91[a]	2.38[a]
	T2	3.80[a]	2.54[a]
	T3	3.92[a]	2.32[a]
	T4	4.21[a]	2.33[a]
20	T1	4.64[a]	2.93[a]
	T2	4.35[a]	3.37[b]
	T3	4.56[a]	3.08[b]
	T4	4.71[a]	2.98[b]

Means followed by the same letter are equal to each other statistically to the Tukey test at 5% probability. MPa: Mega pascal.
Source: Paraguay (2016).

7.29% at 15 cm; and 4.97% at 20 cm, which are considered good or very good being that it is below 15% of the CV in which it is existing as a statistical parameter that indicates, in percentage terms, the dispersion of a series of data with respect to the mean value. According to Pimentel and García (2002), the value of the CV is equal to 0 when there are no differences between the points, resulting in a totally homogeneous distribution. In synthesis, this parameter gives us an idea of the precision of the experiment. Considering the CVs commonly obtained in field agricultural trials, we can consider them good or very good when less than 15%, acceptable when between 15 to 25%, and bad to discard when they are greater than 25%.

With the results obtained in this study, a level of differential soil resistance was found, in which the greater soil depth showed the highest values, related to the compaction than the values found at 10 and 15 cm.

According to Nacci and Pla (1992), the highest values of resistance to penetration take place between 15 and 25 cm deep, which reaffirms the physical restrictions of the soil for the growth and development of the roots attributed to the effect of conventional management. The same trend is reflected in this study, where the same behavior is noted, and from 15 to 20 cm is the highest level of compaction.

Nesmith et al. (1987) estimated that the critical levels of resistance to plant growth vary according to the type of soil and the cultivated species. The authors indicate that the values 1, 2 and 3.5 MPa, respectively, are the critical thresholds of resistance to root penetration. In general, however, Taylor et al. (1966) expressed that the critical limit value is 2MPa, with these authors showing that the level of compaction of the soil studied is compacted.

Krüger (1996), who observed soils with several years of tillage in Argentina, revealed that after 15 cm of the surface a compacted layer appears, which hinders the radical development of the plants.

Results of the obtained means, where the comparison of the different treatments is made with respect to the variable of resistance to the penetration of the soil measured at the end of the crop cycle (Table 2), confirms that among the evaluated treatments with the depth of 10 and 15 cm, there was no significant effects; however, compared to the data (Table 2), reduction of the level of soil resistance with use of different densities of the

Table 3. Variation in soil moisture content (%) in sowing densities of *Cajanus cajan*. Concepción, Paraguay, 2016.

Treatment	% of soil moisture content	
	Before sowing	Crop final cycle
T1	5.20[a]	8.81[a]
T2	5.40[a]	9.80[a]
T3	4.85[a]	9.15[a]
T4	4.00[a]	8.80[a]
CV %	23.80	46.87
G.M.	4.86	9.14

Means followed by the same letter are equal to each other statistically by the Tukey test at 5%. CV: Coefficient of variation; GM: general means.

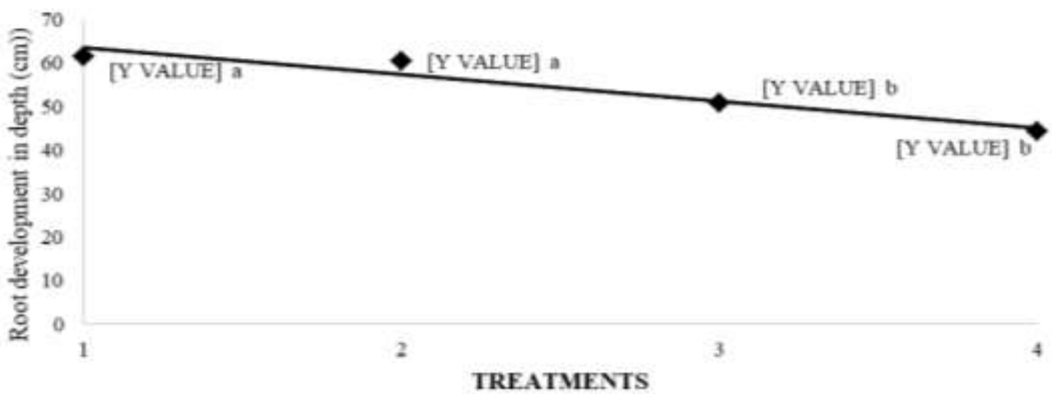

Figure 1. Root development in soil depth according to the densities of *Cajanus cajan*. C.V. = 7.26%. Source: Paraguay (2016).

biological material (*C. cajan*) is considerably noticeable. The T2 at 10 cm has a difference of 1.68 MPa, whereas at a depth of 20 cm, there are significant differences; the lower soil resistance shows T1, being T2, T3 and T4 similar to each other, demonstrating the positive effect of the use of *C. cajan* in the compaction of the soil.

Forsythe et al. (2005) determined that the critical value of penetration resistance for maize crop yield is 2.75 MPa. The same author also reports the value of 2.96 MPa as a limiting value for the growth of the roots. In this work, it was possible to obtain 2.93 MPa at 20 cm depth, which agrees with the mentioned authors, thus implying that the biological material used corrected to a great extent the initial compaction of the soil of the plot studied.

Soil moisture percentage

Contrasting the data obtained in the determination of soil moisture percentage carried out together with the determination of resistance to soil penetration, the average of 4.85% soil moisture was found. At the end of the crop cycle, it can be noticed that the moisture of the soil amounted to 9.15%, observing the effect of the coverage of the fallen leaves of *C. cajan* (Table 3).

Soil moisture, as well as soil depth, has direct and indirect influence on the results in terms of resistance to penetration in the different management systems (Orzuza, 2010).

Soil compaction is directly related to the moisture and depth of the soil as can be seen in the results obtained, where the higher the percentage of moisture present in the soil, the greater will be its depth leading to decrease in compaction (Myers and Robbins, 1991).

Guillén et al. (2006) found lower values of resistance to penetration even in the months of lower precipitation with average values of 2 MPa. In this research, the percentage of moisture found agrees with the authors', since in all the depths, the indicated average were significantly exceeded, implying the direct relationship of soil moisture with soil compaction.

Root development

Analysis of Figure 1 revealed that T4 had less depth in the development of the roots compared with the other treatments whereas T1 presented the best value with

61.56 cm. This implies that there is a close relationship with the soil moisture mentioned earlier.

The root distribution for the density of 200,000 pl ha^{-1} (T4) was the one with the lowest value, and with T1 being the best, the development of the roots reached an average of 61.56 cm in depth, being thicker, deeper and where better effect was obtained in the compaction of the soil, far surpassing the other treatments mentioned in Figure 1.

Spek and Purnomosidhi (1995) mentioned that the competition for water and nutrients depends on the relative distribution of the fine roots of the plants; however, it must be taken into account that this competition occurs with the vegetation that is in its base and has to do with the diameter and depth of these roots. During determination of this effect, it can be noticed that when increasing the plant density of the crop, development of the roots diminished remarkably due to the competition for nutrients.

Rojas (2000) found no significant differences in soil moisture content. In addition, the distribution of roots varied significantly between the different depths, which coincide with what was found in this study.

CONCLUSIONS AND RECOMMENDATIONS

The different densities used in the present experiment revealed positive results in resistance of the soil to penetration in the three depths, which could penetrate and break the surface with the soil moisture percentage becoming better with high density (200,000 pl ha^{-1}) while in depth root development has been found to influence the low density (60,000 pl ha^{-1}).

The initial compaction of the soil is at a high level in the three depths that were measured; at 10 cm the level found was 3.63 Mpa, at 15 cm it was 4.21 Mpa, while for 20 cm the level was 4.71 Mpa with everyone above the critical level (2 Mpa).

The compaction at the end of the crop cycle in the three determinations made shows that the biological material used (*C. cajan)*, had positive effects since it could penetrate and break the surface, solving the problem of the initial compaction mentioned. Here, for the 10 cm depth, the level found was 1.68 MPa, at 15 cm it was 3.32 MPa whereas at 20 cm the level was 2.93 MPa.

The soil moisture percentage had an average of 4.85% at the beginning of the implementation of the crop and with 9.15% at the end of the crop.

The greatest depth of the root was found in the case of T1 with 61.56 cm, greatly surpassing the surface of the plowing foot.

It is also recommended that the research be continued to obtain reliable results so that the producers, when accessing the information, can obtain relevant data to select the suitable practices when faced with a considerable degraded and compacted soil and thus proceed to its adequate recovery.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment of pre and post-harvest management practices on coffee (*Coffea arabica* L.) quality determining factors in Gedeo zone, Southern Ethiopia

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In Gedeo zone, coffee quality is declining from time to time due to several improper pre and post-harvest management practices. Therefore, this study was designed with the objective of assessing impact of pre and post-harvest management practices on *Coffea arabica* L. quality determining factors in Gedeo zone, Southern Ethiopia. Totally, 90 household respondents were used from three Woredas and selected purposively. Finally, quantitative data was analyzed by employing SPSS (version 20). In pre harvesting practices, the result indicated that most of the respondents owned old coffee trees (>20 years), prune their tree frequently within one year interval and observed various diseases on their coffee tree. According to the survey result, majority of farmers use shade trees, which is the most common cropping practiced by coffee producing farmers in the study area, and majority of the farmers use dry method of coffee processing. Descriptive statistics of the field survey revealed that majority responded the provision of support and training from the respective Agricultural research and Development offices. On the contrary, the coffee farming family suffered from shortage of money as well as time at harvesting stage of coffee. On the side of traders, they received extension services in maintaining coffee quality in particular. Most of the traders in the study area in order to buy and sell coffee, did not get advises from market advisers. In general, most management practices in addition to trading methods in the study area have problems in maintaining coffee quality in the zone.

Key words: *Coffea arabica* L, coffee quality, pre-harvest, post-harvest, Gedeo zone.

INTRODUCTION

Coffee (*Coffea arabica* L.) is a non-alcoholic stimulant beverage crop that belongs to the family *Rubiaceae* and genus *Coffea.* Coffee is the world's favorite drink (Techale et al., 2013). Coffee Arabica is believed to

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originate in humid high rain forests of south and south western Ethiopia. Coffee ranked as the fifth most important trade commodity after wheat, cotton, maize and rice (FAO, 2008).

Ethiopia is known for producing the finest Arabica coffee to the world market (Herhaus, 2014); however, the deterioration of the quality of coffee produced is a major challenge in the country (Birhanu et al., 2013). Coffee is the major source of foreign currency income for Ethiopia and contributes more than 35 percent of total export earnings (Feyera, 2006). Ethiopia is well known not only for being the home of Arabica coffee, but also for its very fine quality coffee acclaimed for its aroma and flavor characteristics. Coffee is distinguished based on unique characteristics which includes Sidamo, Yirgachefe, Harar, Gimbi, Jimma and Limmu coffee types (Anwar, 2010). On the other hand, coffee produced in some parts of Ethiopia, especially from Harrar and Yirgachefe, is always sold at a premium price both at domestic and international coffee markets because of its distinctive fine quality (Chifra et al., 1998; ITC, 2002) and appropriate processing approach. Ethiopia possesses a diverse genetic base for the Arabica coffee with considerable heterogeneity and is the center of origin for Coffee arabica, even though Ethiopia produces a range of distinctive Arabica coffees and has considerable potential to sell a large number of specialty coffee (Gebreselassie et al., 2017).

However, in Ethiopia, the quality of coffee produced by farmers has been deteriorating from time to time. Moreover, factors that determine coffee quality are genotypes, climatic conditions, and soil characteristics of the area, agronomic practices, harvesting methods and timing, post-harvest processing techniques, grading, packing, storage conditions and transporting, all contribute either exaltation or deterioration of coffee (Behailu and Solomon, 2006). According to Duguma et al. (2018) at the pre-harvest level, inadequate use of fertilizers, limited moisture, lack of practicing rejuvenation and pruning, coffee wilt and berry diseases, insect pest incidence were the main factors to deteriorate the quality coffee production as well as at the post-harvest level, carrying out of improper harvesting practices, hardly use of recommended packaging materials, conducive storage system, mixing of water and foreign matters on dried coffee were some of the factors affecting the quality of the coffee. Also harvesting and post-harvest, processing methods highly significantly influenced all coffee quality parameters (Ameyu, 2017).

Coffee is significantly produced in four Gedeo zone districts (Dilla Zuria, Wenago, Yirgachefe and Kochere) and serves as a major means of income for the livelihood of coffee farming families. Despite the favorable climatic conditions, variety of local coffee types for quality improvement and long history of its production in Gedeo zone, coffee quality is declining from time to time due to several improper pre and post-harvest management practices.

However, in the Yirgachefe zone, coffee always sold at a premium prices both at domestic and international coffee markets because of its distinctive fine quality and appropriate processing approach, but the other coffee producing districts in the zone do not have same quality like that of Yirgachefe . Still, there are gaps such as lack of profound assessment works to estimate the prevalence of coffee quality problems in different districts of Gedeo zone, lack of adequate information on the effects of post-harvest and pre-harvest handling techniques on coffee quality.

So it is must to give an attention for those practices because they affect directly or indirectly raw bean as well as liquor coffee quality parameters under various stages. Therefore, the study was designed to assess impact of pre and post-harvest management practices on coffee (*C. arabica* L.) quality determining factors in Gedeo zone, southern Ethiopia.

MATERIALS AND METHODS

Description of the study area

This assessment work was conducted in Gedeo zone, southern Ethiopia in a year (2016-2017). Gedeo zone is one of the 13 zones of Southern Nations and Nationalities Peoples Regional State (SNNPRS) of Ethiopia; it has six rural districts (Dill Zuria, Wenago, Yirgachefe, Kocherie, Bule and Gedeb). Gedeo lies between 5 and 7° North latitude and 38 and 40° East longitude. Gedeo extends south as a narrow strip of land along the eastern escarpment of the Ethiopian highlands into the Oromia Region, which borders the zone on the east, south and west; Gedeo shares its northern boundary with Sidama zone. The area characterized as warm humid temperature with mean annual temperature ranges between 17 and 22.4°C and mean annual rainfall between 1200 and1800 mm. The area altitude ranges from 1200 m.a.s.l in the vicinity of Lake Abaya to 2993 m.a.s.l at Haro Wolabu Pond, Bule woreda.

Sampling technique and procedure

At first stage, from six districts of Gedeo zone, 4 districts (Dill Zuria, Wenago, Yirgachefe and Kochere) produce coffee but by considering limiting factors such as time, money and other facilities; 3 districts (Wenago, Yirgachefe and Kocherie) were selected purposively due to the fact that these districts are well known in coffee production in the zone, and also, the crop is significantly produced and serves as a major means of income for the livelihood of coffee farming families. Then by considering the size and suitability of the districts, 3 kebeles were selected purposively. From each kebeles based on amount of coffee production, 5 PAS (peasant associations) were selected purposively from each kebeles, then 6 highly producer household farmers were selected purposively from each selected PAS. The list of household heads in the selected PAS was obtained from the kebele offices and development agents (DAs). Finally, the sample frame of the study were made up of the total number (90) of respondents from the selected districts and interviewed to point out their views based on the questioner in collaboration with local administers, key informants and DAs. Besides, coffee traders (47) were selected purposefully from the three districts with the collaboration of kebele offices and DAs and interviewed to point out their views based on the questionnaire.

Methods of data collection

A semi-structured questionnaire with both close and open ended question was set to collect primary data. Secondary data and information were also collected. To develop the questionnaire (to collect information), in-depth interviews were conducted with farmers, government office, non-government offices, DAs, local administers and key informants. Then, coffee producers and traders were interviewed to point out their views on pre and post-harvest management practices as well as related problems on coffee quality in the Gedeo zone. Additionally, focus group discussions were held with farmers to strengthen and cross-check the data obtained from different stakeholder in selected survey areas. The survey was supplemented by experts' knowledge, cooperative unions, kebele offices and DAs.

Analysis of field survey

Quantitative data collected from the field through structured questionnaires were analyzed by employing the statistical procedures of SPSS version 20 software. Percentage mean, frequency distribution, proportion and ratio were used to analyse the qualitative data. Qualitative data gathered from various sources was organized, triangulated, interpreted, discussed and narrated. Problem ranking was done to identify the magnitude of different factors which are affecting coffee quality in study the area.

RESULTS AND DISCUSSION

This study was undertaken under field condition. The field survey data were collected from respondents using structured questionnaire.

Analysis through descriptive statistics

Demographic factors

Table 1 presents demographic and socioeconomic characteristics of the sample respondents. The total sample size of farm respondents handled during the survey was 90. Of the total sample respondents, 26.7% were male-headed households and only 2.2% were female-headed in Wenago Woreda, 30% were male-headed households and 1% was female-headed in Yirgachefe Woreda and also, 37.8% were male-headed households and only 2.2% were female-headed in Kochere Woreda. Totally, 95.9% of respondents were males and 5.1% were females in the survey areas. This result confirmed the prior expectation that male-headed households have more access to improved technology, updated information, credit and extension services than female-headed household. This result is consistent with other findings; Doss and Morris (2000) showed that females have less access to an improved agricultural technologies and extension services, which contribute to lower adoption rates. In addition to, male-headed households have better access to information than female households do, which helps for adoption of improved agricultural technologies. Therefore, it said to be on improving and maintaining coffee quality male-headed, household can have better than female-headed.

About 46.7% of the respondents were found in the age category of greater than 36 and less than 50 years, in the age category of greater than 50 years 20%, and greater than 25 and less than 35 years 16.7%, while the remaining 6.6% were greater than 10 and less than 25 years old as shown in Table 1. From three survey areas, high numbers of farmers were categorized under age of greater than 36 and less than 50 in Wenago and Kochere areas. But in Yirigachefe woredas, majority of farmers were found to be in the age category of greater than 50 years.

With regards to educational status, majority of respondents who are living in Kochere were found to be educated which is 36.7% compared to other woredas and 5.6% of Yirgachefe respondents were illiterate. Educational status of the household head in the three woredas has significant difference at 5% significance level. Level of education can have a significant effect on coffee quality because illiterate farmers are not well adopting improved technology. Similarly, the finding of several studies revealed that the level of education is a strong and important determinant of farmers' adoption of improved agricultural technologies (Zemedu, 2004). Besides, as reported by Deressa et al. (2009), years of schooling positively influenced farmers' adoption decisions on improved agricultural techniques. Therefore, education is crucial for the farmers to understand and interpret the information coming from any direction to them.

In terms of family size, the majority of sample farmers (61.1%) had less than ten family members, while 15.6% of them had greater than ten family members. Family size also showed variation at 5 percent significance level.

Pre-harvest factors and some agronomic practices

Age of coffee trees

The result of the field survey showed that among 90 coffee farmers interviewed, 74.4% owned old coffee trees (>21 years), while 25.6 % of them owned coffee trees less than twenty one years old as shown in Figure 1. This result implies that majority of the coffee plantations in the study areas are physiologically declining as their yield and quality might decrease as reported by Clifford (1985). The survey result was supported by Duguma et al. (2018) and most of Chole district coffee is found in the interval of old coffee trees which give a low both in the quality and amount of product. Yigzaw (2005) reported that samples from young trees are likely to be mild and thin, but fine in

Table 1. Demographic and socioeconomic characteristics of samples (categorical variables).

Variable	Item	Wenago		Yirgachefe		Kochere		Total	
		N	%	N	%	N	%	N	%
Household	Female	2	2.2	1	1.1	2	2.2	5	5.1
	Male	24	26.7	27	30	34	37.8	85	95.9
Age	10-25	3	3.3	3	3.3	-	-	6	6.6
	26-35	3	3.3	8	8.9	4	4.4	15	16.7
	36-50	14	15.6	8	8.9	20	22.2	42	46.7
	>50	5	5.6	12	13.3	10	11.1	27	30
Family size	1-5	7	7.7	6	6.7	8	8.9	21	23.3
	6-10	15	16.7	19	21.1	21	23.3	55	61.1
	11-15	3	3.3	6	6.7	5	5.6	14	15.6
Educational status	illiterate	4	4.4	5	5.6	1	1.1	10	11.1
	1-8 grade	15	16.7	19	21.1	24	26.7	58	64.5
	9-12 grade	7	7.7	6	6.7	9	10	22	24.4

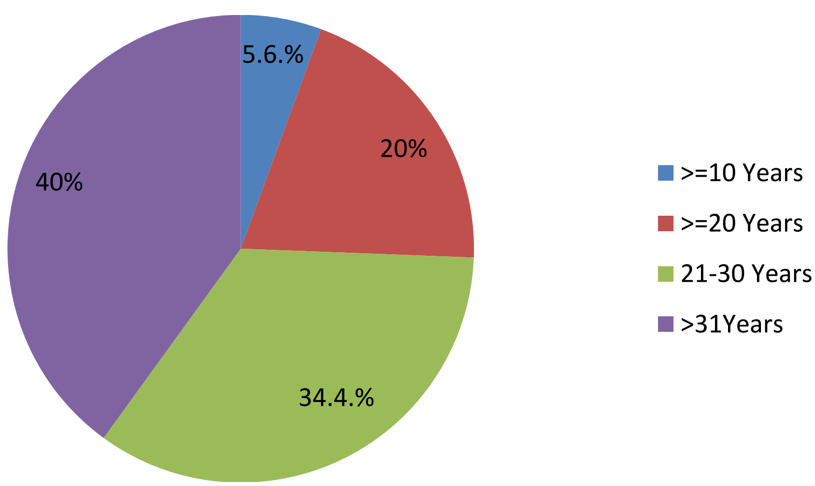

Figure 1. Age of coffee tree in study area owned by respondents.

flavor. Beans from old trees produced strong taste and a harsh characteristic brew. Medium aged trees, 15 to 20 years old bear beans with good flavor as well as acidity and body, thus in this study, most coffee trees aged above 20 years are hypothesized to have been inversely related with coffee quality. In general, poor coffee quality is being produced because of old age.

Coffee tree pruning

In this study, the result showed that 94.9% of respondent coffee farmers practiced pruning. 45.6 and 41.1% of respondents practice formative and maintenance pruning respectively and also 84.4% of respondents prune their tree frequently within one year interval as shown in Table 2. Pruning practice has its own role in the quality of coffee. Goal of pruning is to create well-structured and healthy trees that give good cherry yields over a long period of time or to rejuvenate old trees by stumping. According to Franca and Oliveira (2009), coffee tree pruning is an extremely important pre-harvest activity for reducing incidences of diseases, modifying air movement within the plantation, which in turn reduces leaf drying time and helps maintaining the frame work of the plants in desired shape. In addition, Wintgens (2004) reported that coffee pruning can usually have a positive effect on bean size and flavor.

Table 2. Practices of coffee tree pruning, type and frequency in study area.

Variable		Frequency	Percent
Coffee tree pruning	Yes	85	94.4
	No	5	5.6
Type of pruning	Formative pruning	41	45.6
	maintenance pruning	37	41.1
	Rejuvilination	12	13.3
Frequency of pruning	once monthly	13	14.4
	once per a year	76	84.4
	Other	1	1.2

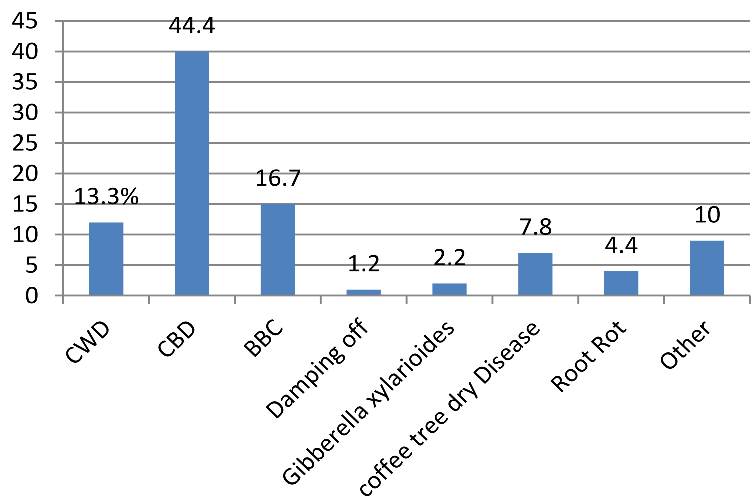

Figure 2. Type of disease in study area in percentage.

Type of coffee disease

It was identified that majority of coffee farmers responded the prevalence of disease like coffee wilt disease (CWD), coffee berry diseases (CBD), BBC, damping off, *Gibberella xylarioides*. From such diseases 44.4% of the farmers mentioned that the area has been widely threatened by coffee beery disease followed by BBC and CWD as shown in Figure 2. As the consequence of this, the quality and quantity of coffee might have decreased considerably. This finding goes in line with the report that mentions CBD, *Colletotrichum kahawae*, CWD, *G. xylarioides*, coffee leaf rust (CLR) and *Hemileia vastatrix* as the major diseases reducing production and quality of coffee in Ethiopia (Zeru et al., 2005). Diseases attack can affect the cherries directly or cause them to deteriorate by debilitating the plants, which will then produce immature or damaged fruits disease and insect attack (such as leaf miner and mites) may also result in lower quality beans and influence the final quality (Gole, 2015).

According to this survey, result showed that 51.5% of respondents use cultural practices to prevent coffee diseases, whereas, the other respondents use other mechanisms to prevent coffee disease (Table 3). The surveys also revealed that farmers of Gedeo zone are not using any type of chemical as control mechanisms. Instead, farmers have some indigenous knowledge and experience to control the diseases. This includes planting coffee trees under shade to reduce transpiration and make them less stressed (not to be easily attacked by the disease) and application of farm yard manure and/or compost. Moreover, frequent cleaning and burning of fallen leaves, fruits and plant debris are methods used by farmers.

Type of coffee weeds and control mechanism

The weed is found to be a serious problem which reduces the productivity and quality of coffee in most areas. As indicated in Figure 3, majority of the coffee plantations in the study areas are infected by soft weed (66.7%), couch grass (7.8%) and a combination of soft weed and couch grass (25.5%). Therefore, it is true that

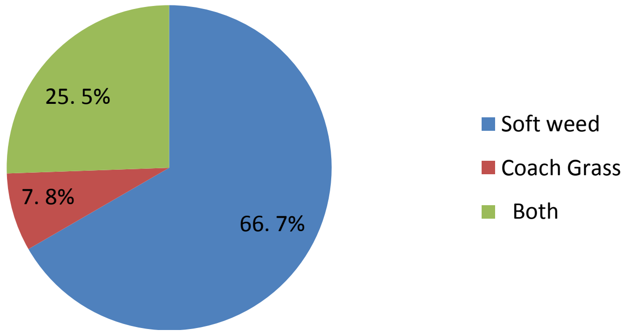

Figure 3. Types of weed present t in the study area.

quality could decrease because of the competition for nutrient, light and moisture with different types of weeds growing in coffee field. Similar findings were reported by Franca and Oliveira (2009). To control this weed problem, the study indicated that most of the respondents used slashing mechanisms (52.2%), whereas, 44.6% of the respondents used hand weeding to control weed and the remaining 2.2% of respondents used IPMS method (Figure 4).

Controlling shade level

Farmers in the study area grow coffee both with and without shade in the field. This survey revealed that 98.9% of farmers use shade tree, whereas, about 1.1% of farmers entirely do not use shade in order to cultivate coffee plant as shown in Figure 5. According to the result, majority of the farmers considered in the study used shade for coffee production which indicates that the farmers are following the right practices. Muschler (2001) indicated that shade improved the appearance of green and roasted coffee beans as well as the acidity and body of the brew, especially for those produced in suboptimal (low altitude) coffee production zones, by promoting slower and balanced filling and uniform ripening of berries. As reported by Geromel et al. (2008), shade tree is one of the main factors responsible for the quality of coffee bean. For example, fruits from coffee grown under shade are characterized by larger bean size than those grown under full sun conditions. Also, they reported that shade has different effects depending on the geographical location of coffee tree.

Harvest and post-harvest factors

One of the main factors affecting quality of coffee is harvesting method. An inadequate method of harvesting is responsible for the widespread failure to maintain the inherent quality of coffee produced in Ethiopia (Alemayehu, 2014), harvesting method highly significantly influenced all raw quality attributes (Ameyu et al., 2017). In addition to harvesting system, maturity affects quality of coffee. According to Bertrand et al. (2006), early picking of red cherries gives the best coffee cup and physical quality of *C. arabica*.

According to the survey, coffees were harvested at full maturity stage by 94.9% of the farmers; whereas, 77.8% of the farmers practiced selective hand picking and 86.9% of the farmers in the study area performed the activity by using both family and daily laborer as shown in Table 4. As indicated by Ameyu et al. (2017), selective method of harvesting was better in producing superior quality beans in all parameters. Therefore hand picking only red cherries is one means for accomplishing high quality of the produce. According to Garo et al. (2016) survey result, striping is much faster than picking only red ripe cherries; by doing so, farmers are harming their coffee quality besides decreasing the potential buds which will result in a good yield in the coming season. Coffee cherries which had contact with ground (soil) resulted in earthy flavor in the final cup taste and also the raw coffee quality was less attractive.

During harvesting practice, costs of laborer indicated by 58.6% of the farmers, and labor force which is mentioned by 38% of the farmers were found to be the main constrains (Table 4). As to the stage of maturity and fruit picking, the right practices were performed which maintain inherent coffee quality. In line with this for *C. arabica* in Costa Rica, early picking of red cherries gives the best coffee as it is mentioned by Bertrand et al. (2006) and such superior quality is achieved as the cherries were allowed to ripen on the tree to full maturity before harvesting (FAO, 2010). In addition to producing high quality coffee, uniformity of selectively harvested beans has advantages for processing (Boot, 2006). Furthermore, Endale et al. (2008) pointed out that low caffeine content was found in bean harvested at immature stage (unripe).

Processing and drying

The chemical composition of green coffee and thus the final coffee quality are correctly determined by the mode of post-harvest treatment, that is, the wet and dry processing. Thus, processing is a crucial activity in coffee production and plays a crucial role in quality determination (Mburu, 1999), and the methods vary in complexity and expected quality of the coffee (Wrigley, 1988).

Both sun-drying as well as wet processing methods are operated in Ethiopia, which accounts for 70 and 30% of coffee produced in the country, respectively (Jacquet et al., 2008). According to Musebe et al. (2007), wet processing method resulted in high mean values for good cup quality (attributes like acidity, body and flavor) and bean physical quality (attributes like odor) as compared to the dry processing method. From the result of Musebe et al. (2007), it can be concluded that wet processing

Table 3. Disease control mechanisms in study area**.**

Controlling mechanism	Frequency	Percent
Quarantine	6	6.7
Sanitation and mechanical	37	41.1
Cultural method	46	51.1
Other	1	1.1

Table 4. Harvesting of coffee in study area.

Variable		Frequency	Percent
Harvesting practice	Selective	68	75.6
	Strip	8	8.9
	Collecting	14	15.5
	Total	90	100
Harvesting stage	Fully maturity stage	85	94.4
	Green stage	3	3.3
	Immature stage	2	2.2
	Total	90	100.0
Constrain during harvesting	Time	2	2.1
	Labor force	37	41.1
	Money to pay for labor	50	55.6
	Others	1	1.2
	Total	90	100.0
Who harvest	Own family	3	3.3
	Daily laborer	10	11.1
	Both	77	85.6
	Total	90	100

method is the best approach to obtain fine and typical quality flavor in the cup that attract consumers according to their preference in the international market.

As indicatde in Table 5, the majority (56%) of the farmers in the study area prepared coffee by using a dry processing method and 32% of farmers used wet processing method. In agreement with this, Alemseged and Yeabsira (2014) mentioned dry processing as the age-old method of processing coffee and is still used in many countries where water resources are limited. According to Ameyu et al. (2017) dry processed method coupled with drying coffee on mesh wire was best in producing coffee beans with high raw quality. In contrast, dry processing using bare ground produced inferior coffee for all raw quality attributes. Drying beds can be made of mesh wire, wood posts, or any suitable local material covered in a material like burlap or nylon netting (Alemseged and Yeabsira, 2014). Most of the farmers (90%) did drying on wooden and bamboo made bed in study area. This shows that the farmers in the study area are exercising the right drying method.

Coffee beans are supposed to be stored with a moisture content of 11–12% which needs to be determined by using moisture tester. However, 94.9% of the farmers in the study area measured moisture content and 98.9% of them use local method (teeth) to determine moisture content. Thus, using local method does not allow measuring the required amount of moisture content in the coffee bean. Drying is considered an important step in quality coffee production since moisture levels higher than 12% can promote microbial growth and mycotoxin formation (Getachew et al., 2015).

Packaging and storing

As indicated in Table 6, farmers in the study area used both jute bags and plastic bags as packing materials. The result however, showed that 67.7% of the interviewed farmers used plastic bags. Such practices are in contrary to the proper packaging which uses jute bags that enable maintaining the inherent quality of coffee.

Table 5. Processing and drying of coffee in study area.

Variable		Frequency	Percent
Method of coffee processing	Dry	49	56.6
	Wet	31	32.3
	Both	10	11.1
	Total	90	100
Drying coffee	On cemented	1	1.0
	On wooden and bamboo	81	90.9
	On ground	8	8.1
	Total	90	100
Checking the moisture content	Yes	85	94.4
	No	5	5.6
	Total	90	100
Method of checking moisture	Local method (teeth)	89	98.9
	Other	1	1.1
	Total	90	100
Time before processing	1-2 days	58	64.4
	3-5 days	8	8.9
	For 1 week	15	16.7
	1 hr	9	10
	Total	90	100

Table 6. Packing and storage of coffee in study area.

Variable		Frequency	Percent
Packing bag	Jute bag	28	31.1
	Plastic bag	61	67.8
	Clay pot	1	1.1
	Total	90	100
Separate storage house	Yes	61	67.8
	No	29	32.2
	Total	90	100
Reason for separate storage house	To avoid contamination	67	74.4
	For have free store	23	25.6
	Total	90	100
Time before marketing	Less than 4 months	30	33.3
	Greater than 4 months	60	66.7
	Total	90	100

Storage is one of the most important and crucial stage in processing of any agricultural commodity. The assessments result showed that 67.8% of the interviewed farmers stored the product in separate storage to avoid contamination as shown in Table 6. Storage facilities should be clean, cool, shaded, dry and well ventilated. In conditions of high relative humidity and temperatures, coffee beans will absorb moisture and develop mold.

Table 7. Transporting and marketing of coffee.

Variable		Frequency	Percent
	Yes	39	43.3
Cash shortage	No	51	56.7
	Total	90	100
	Yes	9	10
Selling at flower stage	No	81	90
	Total	90	100
	Yes	2	2.2
Mix differently harvested	No	88	97.8
	Total	90	100
	Separately	85	94.4
Transportation	With other products	5	5.6
	Total	90	100
	To avoid contamination	79	87.8
Reason for separate storage	Easy to transportation	11	12.2
	Total	90	100

They may be bleached out in color and lose some desirable flavor (Belay et al., 2016).

Among the respondents (Table 6), 60.6% of the respondent stored their coffee for more than four months which worsen coffee quality. In the same token, length of bean storage affects cup quality (Yigzaw, 2005). Similarly, Obiero (1996) reported that storing dried parchment coffee for more than six months resulted in woody flavor, and Wintgens (2004) further described such green coffees as *aged* that may suffer loss acidity. Moreover, the relatively high content of glucose present in dry and wet processed green coffees stored beyond 4 to 5 months decreased markedly (Woelore, 1995).

Transport and marketing coffee

Transportation needs to be done in such a way that involves no contamination. According to the field survey which is indicated in Table 7, 93.9% of the farmers transported coffee bean separately and 80.8% of the farmers mentioned that they are aware of such act in minimize contamination. The result obtained from this study showed that 55% of the farmers in the area don't have shortage of cash that force them to sell coffee at flowering stage; as a result, 86% of the farmers don't sell coffee at its flowering stage. The outcome of the assessment also indicated that 97% of the farmers in the area pointed out that they don't mix differently harvested coffee at the time of selling. In general, transportation and marketing of coffee were done as per the standard of the right practices.

Institutional factors

Descriptive statistics of the field survey study result revealed that from a total of 90 coffee farmers interviewed, substantial number of farmers (78.9 and 65.6%) responded the provision of support (such as improved seeds, raised coffee seedlings, etc.) and training from the respective Agricultural and Rural Development Office (ARDO), respectively as described in Table 8. On the contrary, the study revealed that 71.1% of the coffee farming family suffered from shortage of money at harvesting stage of coffee, whereas only 28.9% responded differently. This might be one of the factors that contribute to the decline in coffee quality due to premature harvesting of coffee to ensure cash sources for their families. This result was in agreement with the findings of Mulugeta (1999), Admassu et al. (2008) and Alemayehu et al. (2008). Mulugeta (1999) reported that access to credit, farm size, supplementary inputs, technical and institutional support like the extension service determine the adoption of technologies to maintaining quality of coffee.

Descriptive statistics of the field survey study result revealed that from a total of 90 coffee farmers interviewed, 70% of them had shortage of time, whereas, 30% of them encountered no shortage of time during peak coffee harvesting period as shown in Table 8. This implies that majority of farmers are not able to harvest their own coffee on time, probably due to other farm activities/overlapping of operations. This result is also in line with the finding of (Anwar, 2010).

Table 8. Institutional factors in study area.

Variable		Frequency	Percent
Support from district agriculture and rural development office	Yes	59	65.6
	No	31	34.4
	Total	90	100
Cash shortage	Yes	64	71.1
	No	26	28.9
	Total	90	100
Time constrains during harvesting	Yes	63	70
	No	27	30
	Total	90	100
Training access	Yes	71	78.9
	No	19	21.1
	Total	99	100

Table 9. Access for training about coffee quality for traders in study area.

Variable		Frequency	Percent
Training access	Yes	42	89.3
	No	5	10.6
	Total	47	100.0
Tanning duration	Before a month	5	10.6
	Before 6 month	12	25.6
	Before a year	30	63.8
	Total	47	100
Market advisor	Yes	17	36.2
	No	30	63.8
	Total	47	100
Reason for lack market advisor	I have not heard	26	55.3
	Am not interested	4	8.5
	It is costly	17	36.2
	Total	47	100

Response of coffee traders

Survey results in the study area revealed that 89.3% of the sampled coffee traders had received extension services in maintaining coffee quality in particular, while remaining 10.6% did not get any type of service. Most traders (63.8 %) had access for such type of trainings before a year and the others (36.2%) before a month or before 6 months. Such types of trainings have roles in maintaining coffee quality.

In study area, in order to buy and sell coffee, 63.8% traders did not get advice from market advisers, whereas 36.2% of respondents had market advisers. As indicated in the study, traders had many reasons about market advisers; 55.3, 36.2 and 8.5% of traders responded they have not heard about it, are not interested and it is costly, respectively, as presented in Table 9.

Coffee producing farmers and traders in the studied Woredas have no coffee moisture testers; hence, both farmers and traders use their sense organs to determine moisture contents of the coffee as shown in Table 10. The result in Table 10 shows that 89.36% respondents determine moisture content by their sense organs which mean by crashing with their teeth, 10.64% test by other

Table 10. Moisture tester users and method of testing in study area.

Variable		Frequency	Percent
	Yes	7	14.9
Moisture taster	No	40	85.1
	Total	47	100
	Crashing with teeth(local method)	42	89.36
Method of testing	Other	5	10.64
	Total	47	100

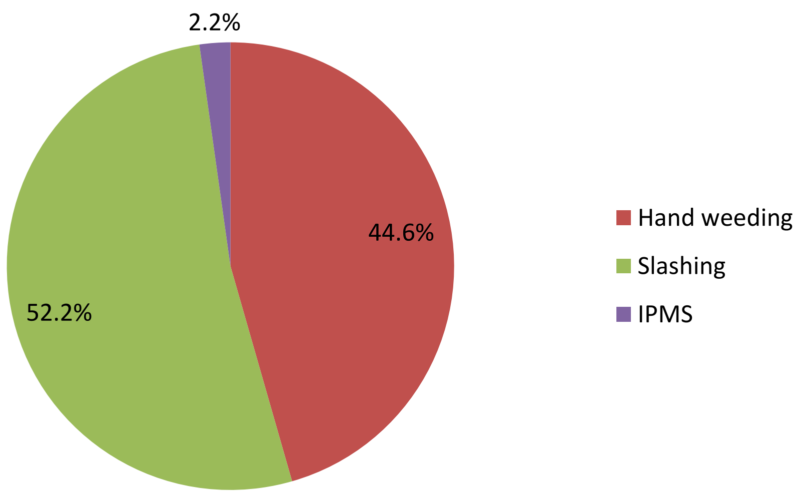

Figure 4. Mechanism used to prevent prevalent coffee weeds in the study area.

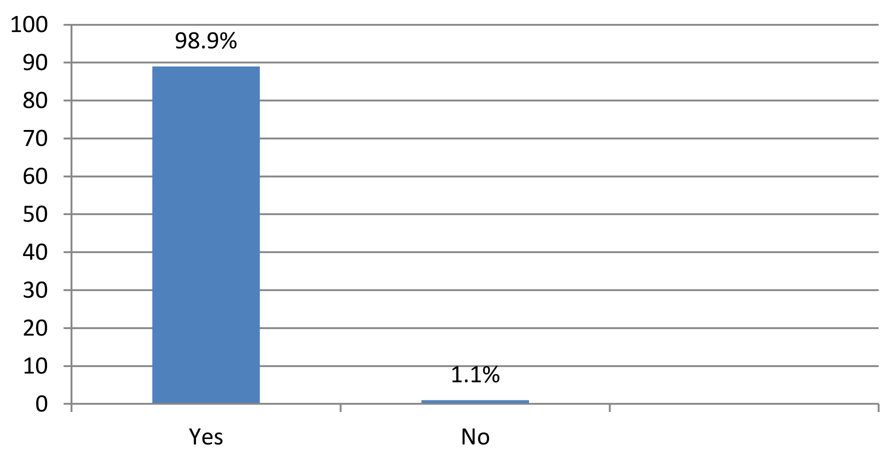

Figure 5. Controlling shade levels in study area.

methods. Drying is considered an important step in quality coffee production, since moisture levels higher than 12% can promote microbial growth and mycotoxin formation (Reh et al., 2006; Getachew et al., 2015).

Conclusion and recommendation

In this survey assessment it can be concluded that like owned old coffee trees, various diseases, plastic bags packaging, storage more than four months, shortage of money at harvesting time and traditional way of coffee moisture testers have their roles in determining the coffee quality under Gedeo zone of coffee production areas. Therefore to overcome the problems affecting the quality of coffee production in the zone, it is better to work with farmers to replace old coffee trees with new ones, and the need for further researches concerning the control mechanism of the diseases. Many researches indicated that wet processing method is preferable to dry processing method; however in the zone, more than half of the processor used dry processing method, therefore, actions should be taken to use wet processing method. Farmers' perception and adoption of technology also have influence on quality of coffee; so, it is important to give different opportunities for the producers to adopt different technologies and give enough awareness for the farmers to change unreal perceptions.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of weed management with glyphosate on growth and early yield of young cocoa (*Theobroma cacao* L.) in Ghana

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Weeds are a limitation to cocoa production, and herbicide use in cocoa cultivation is known to lower the cost of weed management and support better plant growth. As a result, glyphosate was recommended for use in cocoa cultivation following a two-year field trial at the Cocoa Research Institute of Ghana. Recent informal debates among scientists and farmers, however, point towards a disagreement regarding the effect of glyphosate on young cocoa. Consequently, an experiment was conducted from 2011 to 2013 at the Cocoa Research Institute of Ghana to re-evaluate the suitability of glyphosate (Isopropylamine) for weed management and its effects on growth and yield of young cocoa. Five rates of glyphosate viz: (i) 1,920 g active ingredient a.i. ha^{-1} using polyjet nozzle; (ii) 1,920 g a.i. ha^{-1} using very low volume (vlv) nozzle; (iii) 960 g a.i. ha^{-1} vlv; (iv) 720 g a.i. ha^{-1} vlv; (v) 480 g a.i. ha^{-1} vlv; and (vi) manual weeding were tested in a randomized complete block design with four replicates in the field. Effects of treatments on weeds, cocoa growth and yield were recorded for three years. In a gauze house study, cowpea and maize were used to determine residual effects of glyphosate. Emergence, survival and dry matter accumulation by these plants were recorded. Results from the gauze house study showed that glyphosate did not exhibit residual soil activity at these rates. Glyphosate at 960 g ha^{-1} significantly increased yield of three year old cocoa compared to the other rates and manual weeding. The 1,920 g ha^{-1} rates significantly reduced the initial yield of 3 year old cocoa compared to the other glyphosate rates. Cost analysis showed that glyphosate at 960 g a.i. ha^{-1} was Gh¢ 136.00 (11%) and Gh¢ 1,784.00 (61%) cheaper than the 1,920 g a.i. ha^{-1} rates and manual weeding respectively. It was therefore concluded that glyphosate can be applied at 960 g a.i. ha^{-1} (equivalent to 2.0 l ha^{-1} in 120 l of water) for effective weed management without significant adverse effects on growth and yield of young cocoa.

Key words: Weeds, glyphosate, cocoa, growth, bean yield and cost.

INTRODUCTION

Glyphosate is a broad-spectrum, post emergent, systemic and non-selective systemic weedicide (Tu et al., 2001). It has been hailed for its ability to kill annuals, perennials and woody plants while exhibiting favourable environmental attributes (Dale, 2006; Duke and Powles, 2008). Earlier studies suggested that glyphosate neither affected the nervous system nor inhibited cholinesterase activity (Calisle and Trevors, 1988). The tendency of glyphosate to inhibit the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a key enzyme in the shikimate biosynthetic pathway necessary for the production of aromatic amino acids, auxin, phytoalexins, folic acid, lignin, plastoquinones and many other secondary products is core to its weed control properties (Tu et al., 2001; Dale, 2006; Gill et al., 2017). This disruption however, was only reported to occur in plants, fungi and bacteria, suggesting that the rates being used for weed control were not toxic to mammals, birds, fish, amphibians and insects (Franz et al., 1997; Williams et al., 2000). Its overuse however has affected other non-target organisms present in the soil biota (Gill et al., 2017).

Glyphosate translocation in plants occurs in the source-sink direction with up to 70% of absorbed glyphosate translocating out of the treated leaves to the root and shoot apices (Siehl, 1997; Dale, 2006). The translocation process is however, self-limiting and only occurs for up to 48-72 h after application (Dale, 2006). When glyphosate comes into contact with the soil, it is reported to undergo various chemical and physical changes which control its retention, transport and degradation (Duke et al., 2012; Gill et al., 2017).

Glyphosate is partly inactivated by being adsorbed onto soil particles (Al-Rajab et al., 2008) where it is degraded slowly, and partly by rapid degradation of the unbound form through microbial activity (Dick and Quinn, 1995; Tu et al., 2001; Pollegioni et al., 2011; Gill et al., 2017). As a result, glyphosate has low seepage into ground water systems, thus causing minimum contamination (Glass, 1987; de Jonge et al., 2001). It has not shown volatility which will make it an atmospheric pollutant (Duke and Powles, 2008). Because it is soluble in water, glyphosate is not known to accrue in food web (Lane et al., 2012).

The widespread use of glyphosate as a post-emergence weedicide is known to have contributed immensely to increases in the profitability of crop production (Fernandez et al., 2002; Qaim and Taxler, 2005; Sansom, 2012). Used within recommended rates, glyphosate has little or no effects on non-target organisms (Franz et al., 1997). However, excessive use of glyphosate is reported to have adverse effects on metabolic functions of both unicellular organisms (Austin et al., 1991; Zobiole et al., 2011; Shehata et al., 2013; Newman et al., 2016) and wide range of multicellular organisms such as algae (Oliveira et al., 2016), earthworms (Santadino et al., 2014), arthropods (Pérez et al., 2011), honey bees (Balbuena et al., 2015), snails (Druart et al., 2011), fish (Hued et al., 2012), frogs (Pérez -Iglesias et al., 2016, Mann and Bidwell, 1999), lizards (Schaumburg et al., 2016), birds (Oliveira et al., 2007), swine (Lee et al., 2009) and humans (Samsel and Seneff, 2013).

In crop production however, manual weed control during the establishment phase accounts for up to 23% of the total cost (Bonaparte and Toseafa, 1975). Unlike the manual weed control methods, weedicides such as paraquat and glyphosate were found to lower the cost of weed control and resulted in better cocoa growth (Osei-Bonsu et al., 1991; Oppong et al., 1995, 1999). Subsequent field studies in young coffee and cocoa led to similar conclusions (Oppong et al., 2006; Konlan et al. 2014). Both studies went further and reported no adverse effects of glyphosate at the recommended rate of 1.5-2.0 l ha^{-1} on the yield of young coffee and cocoa. In spite of these reports, there still remain concerns about adverse effects of glyphosate; with both farmers and scientist raising red flags regarding the effect of glyphosate on growth and pod production in young cocoa. Some authors have reported that drift during glyphosate application exert adverse effects on fruit and seed formation in other crops through the disruption of aromatic amino acid synthesis, necessary for fruit formation and retention (Magdal et al., 2012; Abella et al., 2013; Salem, 2013). It is possible therefore, that complains by farmers regarding the effect of glyphosate on the cocoa crop holds true. As a response, an experiment was initiated in 2011 using a post-emergent systemic weedicide containing glyphosate, present as 480 g l^{-1} (41.2% w/w) of the Isopropylamine salt for weed management in cocoa. The objectives were to investigate its efficacy as a weedicide, phyto-toxicity and possible effects (immediate and residual) on growth and yield of young cocoa.

MATERIALS AND METHODS

Gauze house study

A gauze house assessment was carried out to determine the residual effect of glyphosate on maize (*Zea mays*) and cowpea (*Vigna unguiculata*). Plastic pots with single drainage holes at the bottom were filled with 2.5 kg top soil each. Two seeds of maize and cowpea were sown separately per pot. The soils in the pots were then watered to field capacity before the application of treatments. Glyphosate was sprayed over these pots at a walking pace as would be done in field application. The control treatment received a spray of water from a clean knapsack. The treatments were arranged in a completely randomized design with 6 replicates. The emergent seedlings were counted at 1 week after sowing (WAS) and were then thinned to one per pot. Seedling survival, plant height and dry matter accumulated were measured at 6 WAS.

Field experiment

Experimental site and treatments

The field experiment was conducted at the Cocoa Research Institute of Ghana (latitude 6° 13' N, longitude 0° 22' W, altitude 222 masl). Treatments were laid out in randomized complete block

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design with 4 replicates. Plot size was 18 x 18 m with cocoa planted at 3 x 3 m. Different rates of glyphosate (i. 480, ii. 720, iii. 960, iv. 1,920 g a.i. ha^{-1} vlv, v. 1,920 g a.i. ha^{-1} polyjet) and vi. manual weeding with a cutlass were used as the treatments.

There were four applications of treatments per year, with each application carried out when weeds were 30 cm tall or 70% ground cover. Other recommended cultural practices necessary for the management of pests and diseases in cocoa were routinely carried out. Data collected from the experimental plots included pre- and post-application weed composition, weekly assessment of weed kill, growth of young cocoa at quarterly intervals and initial yield of 3 year old cocoa trees. Weed kill assessment was done at weekly interval after every application. After four applications in each year, the average annual weed kill was determined by adding each week's assessment and dividing by the number of such weeks in the year. The data was subjected to analysis of variance and differences among treatment means determined by the least significant difference at 5% probability level. Percentage data were arcsine-transformed to fit the assumptions of ANOVA before analysis.

Cost of treatments

Cost components for use of glyphosate included the cost of the weedicide required per hectare for each rate and the cost of labour for application. These were determined as:

$$\text{Weedicide cost/ha} = \text{No. of litres of glyphosate} \times \text{cost/litre} \quad (1)$$

$$\text{Labour cost/ha} = \text{No. of mandays} \times \text{cost/manday} \quad (2)$$

Total cost per hectare per year was then determined as:

$$\text{Cost/ha/year} = (\text{weedicide cost} \times \text{applications/year}) + (\text{labour cost} \times \text{application/year}) \quad (3)$$

The cost for manual weed control comprised the cost of labour and was determined as:

$$\text{Cost of weeding/ha} = \text{No. of mandays} \times \text{cost/manday} \quad (4)$$

$$\text{Cost of weeding/ha/year} = \text{Cost/ha} \times \text{weeding frequency/year} \quad (5)$$

RESULTS

Gauze house study

Post-plant pre-emergence application of glyphosate had no significant ($p<0.05$) adverse residual effects on emergence, survival, growth and by matter accumulated by maize and cowpea plants (Table 1).

Field study

Effects of treatments on weed growth

There were significant treatment effects ($p<0.05$) on percentage weed kill at 2nd, 3rd and 4th weeks after application (WAA) of treatments in the 1st year (Table 2). Manual weeding with a cutlass achieved immediate weed suppression, but weed cover score only showed significant differences ($p<0.05$) between this treatment and the 1,920 g a.i. ha^{-1} (polyjet and vlv) during the 1st and 2nd WAA. The 1,920 g a.i. ha^{-1} vlv rate accounted for the highest weed kill at 8 and 9 weeks after the 1st and 2nd applications (Tables 2 and 3), and also from 3 to 6 weeks after the 3rd application, while the 480 g a.i. ha^{-1} vlv recorded the least weed kill a (Table 4).

The performance of glyphosate at the rate of 1,920 g a.i. ha^{-1} was not significantly ($p>0.05$) affected by the nozzle type (polyjet or vlv) at any time during the three years. There was no significant differences ($p>0.05$) in weed kill among the weedicide treatments after the 1st and 2nd applications. However, the 720 g a.i. ha^{-1} vlv achieved a higher weed kill than the 960 g a.i. ha^{-1} vlv after the 3rd application. Generally, the 720 and 960 g a.i. ha^{-1} vlv achieved higher weed kill after the 2nd and 3rd applications.

Weed species succession

Before treatment application, about 15 to 19 weed species were identified per experimental plot with *Aspillia africana, Justicea sp, Oplismenus burmannii* and *Synedrella nodiflora* constituting the major species (Table 5). The results showed that there was no drastic shift in the weed species composition after the 3 years of repeated application of the treatments. In isolated cases, *Ageratum conyzoides, Amaranthus* spp. and *Euphorbia heterophyla* emerged as species previously absent within the plots.

Growth and yield of cocoa

There were no significant differences ($p>0.05$) in growth (girth and height increase) of young cocoa trees attributable to treatment application (Table 6). The initial yield of the 3 year old cocoa was significantly higher ($p<0.05$) in the 480-960 g a.i. ha^{-1} glyphosate treated plots than in the 1,920 g a.i. ha^{-1} (polyjet and vlv) and the manually weeded plots (Figure 1). There was no significant difference ($p>0.05$) in cocoa yield between the manually weeded plots and plots treated with 1,920 g a.i. ha^{-1} glyphosate rate (polyjet and vlv).

Cost of treatments

Manual weeding resulted in the highest cost of weed control per hectare (Table 7). Labour cost for the application of all the glyphosate rates were the same. The amount of active ingredient required for each rate was the major factor establishing cost differences

Table 1. Residual effects of glyphosate on the emergence and growth of maize and cowpea.

Glyphosate rate (a.i. g ha^{-1})		Final emergence (%)	Survival at 6 weeks (%)	Height at 6 weeks (cm)	Dry matter per plant (g)	% Dry matter per plant
Response of maize	1,920 poly-jet	66.7 (55.0)	100 (88.2)	67.3	3.1	12.8 (21.0)
	1,920 vlv	62.5 (53.1)	100 (88.2)	69.0	2.8	12.9 (21.1)
	960 vlv	83.3 (66.2)	100 (88.2)	88.1	3.1	14.8 (22.6)
	720 vlv	62.5 (57.0)	100 (88.2)	64.7	2.3	13.9 (21.9)
	480 vlv	91.7 (75.6)	100 (88.2)	88.9	3.2	15.5 (23.2)
	Water only	62.5 (53.1)	100 (88.2)	71.0	3.9	15.1 (22.9)
	F-test	ns	-	ns	ns	ns
	% cv	21.6	-	15.9	24.8	10.6
Response of cowpea	1,920 poly-jet	87.5 (72.5)	100 (88.2)	78.6	3.2	17.9 (25.0)
	1,920 vlv	87.5 (72.5)	100 (88.2)	79.2	3.1	16.8 (24.2)
	960 vlv	91.7 (75.6)	100 (88.2)	85.5	3.0	17.3 (24.6)
	720 vlv	91.7 (75.6)	100 (88.2)	85.8	2.6	16.4 (23.9)
	480 vlv	91.7 (75.6)	100 (88.2)	82.8	2.5	17.2 (24.5)
	Water only	87.5 (76.2)	100 (88.2)	81.5	2.5	19.6 (26.3)
	F-test	ns	-	ns	ns	ns
	% cv	19.5	-	10.4	18.9	8.0

Values in parenthesis are arc sine transformations; a.i. g ha^{-1} (active ingredient in grams per hectare).

Table 2. Response of weeds to glyphosate rates and manual weeding 1-9 weeks after application of treatments in first year.

Glyphosate rate (a.i. g ha^{-1})	Weed kill (%)								
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks
1,920 poly-jet	58.2(49.7)	65.5(54.0)	67.8(55.4)	74.0(59.3)	72.2(58.2)	69.0(56.2)	67.2(55.1)	60.8(51.2)	45.0(42.1)
1,920 vlv	58.2(49.7)	70.0(56.8)	75.0(60.0)	83.2(65.8)	81.8(64.8)	78.2(62.2)	75.5(60.3)	67.5(55.2)	55.5(48.2)
960 vlv	48.0(43.9)	54.8(47.8)	58.5(49.9)	70.5(57.1)	67.8(55.4)	63.2(52.7)	59.0(50.2)	46.0(42.7)	33.5(35.4)
720 vlv	51.2(45.7)	55.0(47.9)	57.8(49.5)	59.8(50.7)	53.5(47.0)	49.8(44.9)	47.0(43.3)	44.5(41.8)	37.0(37.5)
480 vlv	47.5(43.6)	51.2(45.7)	52.8(46.6)	52.8(46.6)	46.8(43.2)	42.0(40.4)	37.8(37.9)	27.8(31.8)	21.3(27.5)
Manual control	100(88.2)	85.8(67.9)	78.8(62.6)	75.0(60.0)	67.5(55.2)	63.2(52.7)	60.5(51.1)	27.5(31.6)	6.8(15.1)
F-test	14.6	13.8	14.6	16.3	17.4	17.1	17.5	25.4	20.3
% cv	16.6	14.5	15.0	15.7	17.7	19.2	19.9	25.4	38.4

Values in parenthesis are arc sine transformations; a.i. g ha^{-1} (active ingredient in grams per hectare).

Table 3. Response of weeds to glyphosate rates and manual weeding 1-10 weeks after application of treatments in the second year.

Glyphosate rate (a.i. g ha^{-1})	Weed kill (%)									
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks
1,920 poly-jet	39.5(38.9)	51.5(45.9)	56.2(48.6)	65.0(53.7)	74.0(59.3)	80.8(64.0)	78.5(62.4)	77.0(61.3)	86.2(68.2)	88.2(69.9)
1,920 vlv	67.8(55.40)	75.0(60.0)	77.5(61.7)	80.0(63.4)	83.5(66.0)	90.5(72.1)	89.8(71.4)	89.0(70.6)	92.8(74.4)	93.5(75.2)
960 vlv	44.2(41.7)	50.0(45.0)	62.2(52.1)	72.0(58.1)	77.0(61.3)	87.5(69.3)	87.8(69.6)	86.2(68.2)	86.5(68.4)	87.8(69.6)
720 vlv	37.5(37.8)	52.5(46.4)	55.8(48.3)	66.5(54.6)	72.5(58.4)	82.0(64.9)	81.2(64.3)	79.8(63.3)	83.5(66.0)	84.5(66.8)
480 vlv	30.7(33.7)	35.2(36.4)	42.5(40.7)	46.8(43.2)	51.0(45.6)	57.5(49.3)	55.0(47.9)	52.5(46.4)	51.8(46.0)	53.0(46.7)
Manual control	96.5(79.2)	85.0(67.2)	78.0(62.0)	73.8(59.2)	70.5(57.1)	62.2(52.1)	60.8(51.2)	59.5(50.5)	58.5(49.9)	57.5(49.3)
F-test	15.8	16.3	12.7	12.6	11.4	15.1	15.6	15.2	17.7	20.0
% cv	19.9	18.6	13.6	12.4	10.5	13.0	13.7	13.6	15.4	17.3

Values in parenthesis are arc sine transformations, a.i. g ha^{-1} (active ingredient in grams per hectare).

Table 4. Response of weeds to glyphosate rates and manual weeding 1-6 weeks after application of treatments in the third year.

Glyphosate rate (a.i. g ha^{-1})	Weed kill (%)				
	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
1,920 poly-jet	28.8 (32.5	62.5 (52.2)	79.0 (62.7)	89.8 (71.4)	93.2 (74.9)
1,920 vlv	18.5 (25.5)	77.0 (61.3)	91.0 (72.5)	97.0 (80.0)	98.8 (83.7)
960 vlv	22.0 (28.0)	39.5 (38.9)	55.5 (48.2)	70.0 (56.8)	78.0 (62.0)
720 vlv	35.8 (36.8)	77.2 (61.5)	89.0 (70.6)	92.0 (73.6)	94.5 (76.4)
480 vlv	18.2 (25.3)	20.2 (26.7)	34.5 (36.0)	39.0 (38.7)	45.2 (42.3)
Manual control	100.0 (88.2)	98.0 (81.9)	95.5 (77.8)	85.8 (67.9)	80.8 (64.0)
F-test	16.3	28.5	25.2	16.3	15.2
% cv	30.2	30.6	22.5	13.8	12.3

Values in parenthesis are arc sine transformations; ai g ha^{-1} (active ingredient in grams per hectare).

between rates of application in the glyphosate treatments. As a result, the cost of weed control per hectare per year using glyphosate increased with increasing dosage. Glyphosate at the rate of 1,920 g a.i. ha^{-1} (polyjet and vlv) had the highest weed control cost. Although there were differences in the cost of weed control among the 480, 720 and 960 g a.i. ha^{-1} glyphosate rates, these differences were marginal compared to the higher rates (1,920 g a.i. ha^{-1}) and manual weeding.

DISCUSSION

Effective and timely weed control is critical to obtaining the potential yield in cocoa. The effectiveness of the 1,920 g a.i. ha^{-1} vlv rate during the early periods following treatments application was due to the higher concentration of the active ingredient which facilitated rapid weed kill. Its effectiveness however, declined with time since glyphosate is not known to exhibit residual activity at recommended rates (Franz et al., 1997). This was confirmed by the bioassay results

Table 5. Species composition of weeds in experimental plots before (August 2011) and after (October 2013) treatment applications.

Weed species	1,920 g a.i. ha^{-1} poly-jet		1,920 g a.i. ha^{-1} vlv		960 g a.i. ha^{-1} vlv		720 g a.i. ha^{-1} vlv		480 g a.i. ha^{-1} vlv		Manual weed control	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Paspalum spp.	2.2	*	4.5	*	6.0	*	3.5	*	2.3	*	1.8	0.5
Rottboellia granularis	3.0	6.3	2.3	4.1	1.6	3.5	1.0	2.7	1.5	1.8	10.8	3.8
Synedrella nodiflora	10.5	13.1	29.3	31.7	29.2	26.1	9.0	10.6	43.0	27.8	11.5	15.7
Justicea spp.	25.3	*	8.5	*	17.0	*	10.4	*	22.0	21.4	12.0	5.1
Oplismanus burmannii	17.0	*	10.1	*	5.8	*	32.5	*	11.0	*	22.3	8.3
Chromolaena odorata	4.3	*	2.1	*	4.9	*	2.8	*	4.3	1.7	7	1.7
Amaranthus spp.	10.0	15.7	*	7.5	0.5	8.7	*	16.4	*	11.7	*	7.8
Sapplings	10.2	11.5	2.6	8.1	4.5	7.3	8.3	10.4	12.3	11.3	22.3	*
Ageratum conyzoides	*	3.7	2.0	4.2	*	1.5	0.5	3.1	*	2.5	*	0.8
Commelina diffusa	4.4	*	3.8	*	4.0	2.1	1.0	*	2.1	3.8	1.3	2.7
Sedges	*	1.8	2.0	3.1	1.0	2.7	1.0	*	2.0	2.6	0.8	1.8
Centrosema pubescens	1.3	*	0.5	*	4.3	3.1	*	2.5	0.5	1.2	*	1.7
Desmodium spp.	1.5	*	4.2	*	6.5	*	2.6	3.4	2.0	6.7	10.3	*
Euphorbia heterophylla	*	6.7	*	5.1	*	6.0	*	2.7	*	1.5	*	*
Digitaria insularis	1.0	1.2	*	*	*	*	1.0	*	0.5	*	0.5	0.5
Spigia anthelmia	*	*	*	*	*	*	*	*	*	*	0.5	3.1
Momordica charantia	1.5	3.1	0.5	2.7	1.5	1.5	1.3	*	0.5	*	1.0	5.2
Lantana camara	*	*	5.0	1.7	5.0	*	0.5	*	0.5	*	10.0	*
Aspillia africana	31.5	10.5	49.0	7.8	24.0	5.1	8.5	0.5	7.2	1.7	*	1.2
Phyllanthus amarus	0.5	2.5	*	2.7	2.0	1.2	*	1.5	0.5	2.8	2.0	*
Richardia spp.	50.0	*	0.5	*	*	*	*	*	*	*	1.0	0.8
Chloris batata	*	*	*	*	1.0	*	*	*	*	*	3.0	2.7
Setaria barbata	*	*	*	*	*	*	1.0	*	*	*	0.5	*
Sida acuta	*	*	*	*	*	*	1.0	*	*	*	0.5	*
Panicum maximum	*	*	*	*	*	*	1.0	*	*	*	*	*

Note: *weed species absent.

which indicated the absence of residual effects of the weedicide on emergence and subsequent growth of cereals and legumes, similar to findings of Konlan et al. (2014).

The performance of the lower rates of glyphosate treatments compared to the manual weeding with regard to weed control confirm earlier reports that weedicides ensure better weed control than manual weeding in cocoa (Osei-Bonsu et al., 1991; Oppong et al., 1995; 1999; Konlan et al., 2014). These rates were even more effective when applied to young and fresh weed re-growth, suggesting the importance of proper timing of weedicide application for good results (Wendy et al., 2001).

Table 6. Growth response of young cocoa to glyphosate rates and manual weeding 1-12 months after transplanting and application of treatments.

Glyphosate rate (a.i. g ha^{-1})		3 months	6 months	9 months	12 months
Girth (mm)	1,920 polyjet	4.7	8.1	12.8	15.2
	1,920 vlv	3.7	6.3	9.9	12.9
	960 vlv	4.3	7.0	10.4	14.4
	720 vlv	4.2	6.6	9.7	13.0
	480 vlv	4.4	7.7	11.6	14.7
	Manual control	4.3	6.8	9.2	12.7
	F-test	Ns	Ns	ns	ns
	% cv	23.6	17.1	17.8	14.8
Height (cm)	1,920 polyjet	16.1	26.4	52.1	73.6
	1,920 vlv	10.7	18.6	43.7	63.3
	960 vlv	14.8	23.1	46.0	67.4
	720 vlv	14.6	22.4	58.3	67.3
	480 vlv	16.7	24.5	51.4	76.2
	Manual control	17.9	22.7	46.2	62.8
	F-test	Ns	Ns	ns	ns
	% cv	32.5	29.3	28.6	21.4

Note: a.i. g ha^{-1} (active ingredient in grams per hectare).

Table 7. Cost of weed control as affected by glyphosate rates and labour requirements.

Rate (a.i g ha^{-1})	Litres/ha/spray	Weedicide cost/ha/spray (US$)	Labour cost/ ha/spray (US$)	Total cost/ ha/trt (US$)	Total cost/ ha/year (US$)
1,920 polyjet	4.0	13.60	48.00	61.60	246.40
1,920 vlv	4.0	13.60	48.00	61.60	246.40
960 vlv	3.0	6.80	48.00	54.80	219.20
720 vlv	1.5	5.10	48.00	53.00	212.40
480 vlv	1.0	3.40	48.00	51.40	205.60
Manual	-	-	96.00	96.00	576.00
Cost of 1 manday = US$8.00					
Cost of 1 litre of Sidasate = US$3.40					
No. of mandasy for weedicide application/ha = 6					
No. of weedicide applications/year = 4					
No. of mandays for manual weeding = 12					
No of manual weeding operations/year = 6					
Exchange rate: US$1 = Gh¢5.0.					

The unexpected higher weed kill by the 720 g a.i. ha^{-1} vlv compared to the 960 g a.i. ha^{-1} vlv was probably due to the presence of perennial weeds in the latter plots (Konlan et al., 2014). Because of the non-selective nature of glyphosate, weed species composition after three years was generally representative of what it was at the onset of treatments application. Except in the case of glyphosate resistance, the application of glyphosate is therefore not expected to lead to a buildup of specific weed species at the expense of others as a result of selective killing.

The absence of differences in stem diameter, which is better indicator of plant growth (Glending, 1966) and height increases suggests that growth of the young cocoa was not adversely affected by the application of glyphosate. In spite of similar morphological growth records, yields obtained from the 480-960 g a.i. ha^{-1} vlv treated plots were higher than yields from the 1,920 g a.i. ha^{-1} (polyjet and vlv) and manually weeded plots, where yields were similar.

This result contradicts earlier reports by Oppong et al. (2006) and Konlan et al. (2014), which indicated no

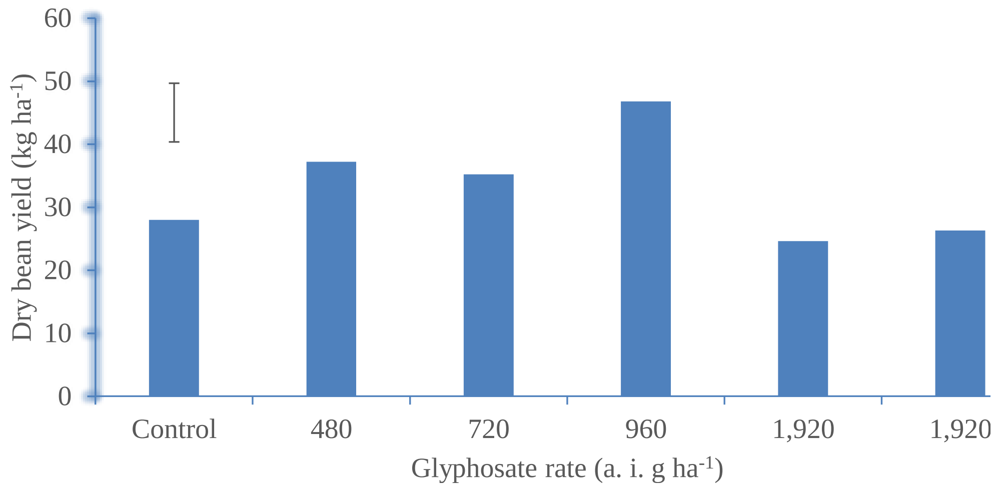

Figure 1. Dry bean yield (kg ha^{-1}) of 3 year old cocoa as affected by glyphosate rates and manual weeding. Note: a.i. g ha^{-1} (active ingredient in grams per hectare), Control (manual weeding).

significant differences in initial yield of young cocoa following glyphosate use for weed control. This may be the result of different field conditions under which the tests were carried out. The lower yields observed in the manually weeded plots and also in the plots treated with the highest glyphosate rates could probably be due to higher weed competition from residual weeds in the manually weeded plots, and strong drift effect after application of the 1,920 g a.i. ha^{-1} glyphosate rates (polyjet and vlv) respectively. The possible disruption of aromatic amino acid synthesis due to the strong drift of glyphosate might have contributed to lower yields since amino acids are known to play a vital role in fruit set and retention (Magda et al., 2012; Salem, 2013).

The cost of manual weed control, which is best recommended for young cocoa, was more than double the cost of the highest glyphosate rate. Dry bean yield (kg ha^{-1}) of young cocoa following manual weed control was comparable to those obtained from the 1,920 g a.i. ha^{-1} glyphosate (polyjet and vlv) treated plots. Lower glyphosate rates (480, 720 and 960 g a.i. ha^{-1}) which were very effective in killing weeds provided cheaper weed control options and increased dry bean yield, thus potentially increasing revenue and net benefits. These lower rates would also exert relatively less adverse effects on soil fauna and also lead to lower drift concentration which has been shown to affect fruit set and retention (Magdal et al., 2012; Abella et al., 2013; Salem et al., 2013). This probably explains why dry bean yields from plots treated with these lower rates were higher.

Conclusion

The yield of three-year-old cocoa in plots that have been treated with glyphosate at rate of 1,920 g a.i. ha^{-1} (polyjet and vlv) for weed control were comparable to those in manually weeded plots. The application of glyphosate at a rate of 960 g a.i. ha^{-1} vlv was cheaper, effectively kept weed growth below economic injury level without immediate or residual adverse effects on the growth of cocoa and consequently, caused improvement in yield above the farmers' practice of manual weed control and other glyphosate rates. It was therefore, concluded that glyphosate can be applied at a rate 960 g a.i. ha^{-1} vlv (equivalent to 2.0 L glyphosate ha^{-1} in 120 l of water) for effective weed control during the establishment phase of cocoa.

Adverse effects of glyphosate on growth and/or yield of young cocoa may however, result from improper application of this rate, effects of strong drift or both. It is therefore not advisable to carry out glyphosate application in windy conditions, as well as during transitioning into flower and fruit production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of the *Papaya´s* maturation degree by electrical impedance

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The economic importance of *Papaya* for fruit producers reinforces the idea of alternative (non-invasive and low cost) strategies to measure fruit ripening. In this work, it has been evaluated the kinetics of ripening degree of *Papaya* based on alternative and non-invasive electrical impedance method that is compared with conventional techniques (mechanical assays, determination of total soluble solids and titratable acidity). It was observed that a single point measurement of real part of impedance (1 MHz) returned relevant information concerning the kinetics of *Papaya* ripening. These results were in agreement with the firmness of fruit (mechanical assays) and the corresponding variation in total soluble solids, providing advantages for identification of ripening degree and depreciation of *Papayas*.

Keywords: electrical impedance, *Papaya*, ripening degree, bulky resistance

INTRODUCTION

The *Papaya* (*Carica papaya* L.) is a product of great importance in the economic scenario of Brazilian fruit. This tropical fruit is the seventh most exported natural fruits in the South America (25 % of world production, with 1.6 million tons per year), considered the largest producer of *Papaya* in the world and the third largest exporter to the USA (El-Gewely, 2008). *Papaya* is a climacteric fruit with high respiratory rate and strong ethylene production after harvest, presenting a high degree of perishability if stored under ambient conditions (Martins et al., 2014). As a consequence, the ripening degree monitoring is a critical process for the post-harvest and following distribution to commercial centers. Conventional chemical and biochemical analyses for fruit ripening determination are based on destructive assays, characterizing a strong drawback to be circumvented (Miloski et al., 2008) since these methods are often laborious and expensive. As a consequence, it is required the developing low cost and non-destructive assays (Islam et al., 2018).

The electrical impedance spectroscopy (EIS) technique has been applied in the evaluation of quality control and

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ripening of fresh fruit such as tomato, banana, cucumber and mango (Souza Junior et al., 2015; Figueiredo Neto et al., 2017). The EIS of nanostructured, organic and biologic systems returns information about charge transport and accumulation at interfaces–electrode/ material and field-dependent mechanisms in structures. These processes are critically dependent on the current pathways created by ionic channels (resistance) and charge accumulation at surfaces (capacitance) can be conveniently measured by direct inspection of the real and imaginary parts of impedance. The transposition of these concepts to the fruit ripening process favors the association of physical parameters with information about physiological and biochemical changes in fruit (Chowdhury et al., 2015). The tissue composition, anatomy and health of fruit compose and determine the complex bioelectrical system (Schwann, 2002) that provide the electrical path for current circulation and charge accumulation. This condition favors the detection of fruit bruising by direct measurement of impedance in anisotropic fruit. Harker and Forbes (1997) reported the use of EIS applied on persimmons (cultivar Fuyu) at 50 Hz for identification of the ripening degree.

Grossi and Riccò (2017) reported the study of impedance changes of mangoes due the ripening process in the frequency range 1 to 200 kHz using a resistor and a capacitor in parallel as an electrical model. The effective resistance presented strong variation at 1 kHz with an increase in the first phase of the ripening process, reaching a maximum after 5 d and then decreasing with further ripening.

If considered that interfacial phenomena are conveniently explored at low frequency range and that bulk properties are explored at high frequency, the ripening degree (that involves primary bulky properties) can be addressed by impedance data measured at the high frequency region. In a previous work of our group (Figueiredo Neto et al., 2017), the ripening stage of mangos was explored from the direct measurement of bulk electrical resistance of samples, normalized by the diameter that provides an intrinsic parameter that is independent on the fruit size.

Based on those important findings, the kinetics of the *Papayas* ripening was evaluated by impedance analysis. Thus, in this paper it is provided the comparison of EIS data with intrinsic physical properties of fruit (such as total soluble solid, loss of weight and mechanical properties) as a function of the ripening stage.

MATERIALS AND METHODS

Samples preparation

The experiments were carried out at the agricultural experimental field of the Technology and Social Science Department of the Bahia State University/DTCS of the State University of Bahia/UNEB, located in Juazeiro, BA, 09°24' latitude and 40°30' WGr longitude and altitude of 368 m. All the samples were collected during early morning from May to August 2018 in Vertissolo soil. The climate of the region is semi-arid according to the Koppen classification. The meteorological data of the area were collected (each week) during the period of the experiments and these are scrutinized in Table 1.

The collected *Papayas* were taken to the Post-Harvest Technology Laboratory of the Federal University of the São Francisco Valley in cardboard boxes with four fruit in each one. The *Papayas* (average weight of 0.4 kg) were washed and dried before the procedure for storage for 12 days at 25 ± 2°C/(65 ± 3) %RH.

After harvest, the samples were taken to the laboratory and immediately weighed individually to obtain the fresh mass (FM). In order of obtained the dry mass (DM) of *Papayas*, the fruit were sectioned in small pieces and placed to dry in a forced ventilation oven with a temperature of (65 ± 5)°C. The fruit were kept in an oven until constant weights were obtained. The drying time was fixed in 24 h. After drying, the fruit were rested in airtight plastic boxes to cool and avoid additional variation of weight.

Characterization techniques

The physical assays, titratable acidity and total soluble solids, were evaluated using three to five fruit per day (during overall ripening stage). The fruit were pulped and mixed to obtain a homogeneous sample and evaluated in triplicate.

The absorbance index was acquired by a DA-meter® – (DA - index of the state of maturation of the fruit) (Turoni/Italia), calculated by the difference between the measured absorbance at 670 and 720 nm (chlorophyll peak) (Noferini et al., 2009).

The measurement of total soluble solids (TSS) was obtained by homogenizing the pulp. Then, a drop (10 ∟ L) of the resulting solution was placed on the lens of an Atago digital refractometer model PR 201 allowing the direct determination of TSS (%). Titratable acidity (TA) assays were evaluated by titrating an aliquot (10 mL) of *Papaya* juice with 0.1 M NaOH solution in the presence of phenolphthalein until pH 8.0 was obtained, providing the results in mg of citric acid per 100 mL of juice (AOAC, 1992).

The water content of the fruit was determined by direct subtraction between FM and DM. The results are presented as water weight in grams (g) and percentage of water in the fruit (%).

The electrical characterization of samples was performed in a Potentiostat/Galvanostat Metrohm Autolab AUT302N applying an AC voltage of 100 mV and a frequency range from 1 Hz to 1 MHz. The electrical contact with the fruit surface was established by using two electrodes of Ag/AgCl (MedLevensohn) disposed on opposite side of fruit (equatorial zone of samples) as shown in Figure 1.

The compression test was performed in an electromechanic universal machine EMIC model DL 10000 and registered by a computer furnished with a TESC software version 3.04 adapted for experiments with agricultural products. *Papayas* (at different ripening stage) were disposed between parallel plates (diameter 30 cm) and subjected to a compression of 5 mm min^{-1} until the final strength was reached. The complete epicarp disruption, providing a direct relationship between force (N) and deformation (mm) of the samples.

The modulus of elasticity (E) of the papayas was calculated according Equation 1 that used the Hertz model, applied in the determination of the maximum surface pressure and elastic modulus of a body pressed between two flat plates (Khodabakhshian and Emadi, 2011).

$$E = 0{,}75F(1-\mu^2) * \frac{1}{D^{3/2}} * \frac{1}{R^{1/2}} \quad (1)$$

Table 1. Average of air temperature (T), relative humidity (RH) and precipitation (p), from May to August 2018 measured every morning in the corresponding period.

Month	T (°C)	RH (%)	p (mm)
May	25.7	59.1	17.3
June	24.7	58.7	3.3
July	24.5	53.7	0.5
August	25.3	18.6	1.0

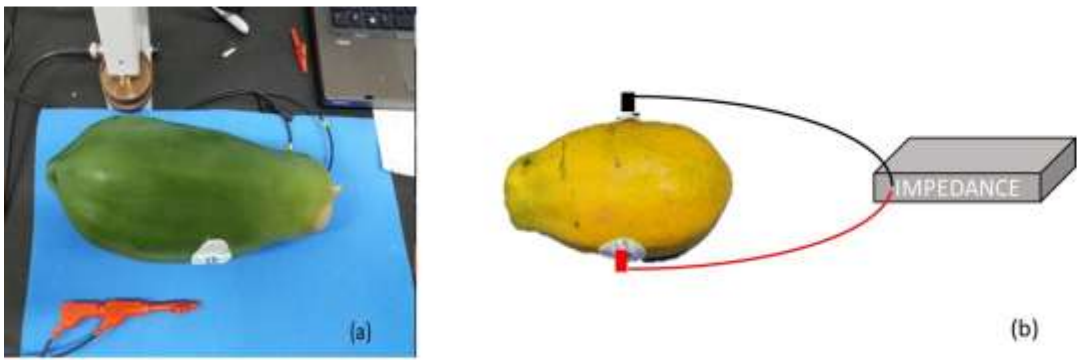

Figure 1. Disposition of electrodes for electrical characterization of samples at different ripening degree (a) 0 d and (b) 4 d- with corresponding connections to impedance analyzer.

where, F is the force in Newton, ∟ is the Poisson's ratio, R is the radius of the theoretical sphere (m), representing the fruit and D is the deformation (m).

Hencky's stress (σ_H) and strain (ε_H) were determined by Equations 2 to 4, as reported by Costa et al. (2017), Ferrari et al. (2011) and Linares et al. (2013). The breakdown stress is calculated considering the peak value of the stress-strain curve (Equations 1, 2 and 3).

$$\sigma_H = \frac{F(t)}{A(t)} \quad (2)$$

$$\varepsilon_H = -\ln\left(\frac{H(t)}{H_0}\right) \quad (3)$$

$$A(t) = \frac{A_0 H_0}{H(t)} \quad (4)$$

where, σ_H is Hencky stress (N m^{-2}); ε_H is Hencky strain (%); F(t) is Force (N) as a function of time t (s); A is Area (m^2) versus time (s); A_0 is initial sample area (m^2); H_0 is initial sample height (m) and H(t) is sample height (m) versus time (s).

Five groups of experiments were performed at different period of time after harvest (0, 2, 4, 6 and 8 d) considering four fruit per day. As a consequence, each point in experimental data refers to the average of 4 assays in different fruit. The error bar expressed in Tables and Figures was calculated from measurement in quadruplicates.

RESULTS AND DISCUSSION

Beyond the typical modification in parameters such as total soluble solids, titratable acidity, elasticity and water concentration, there are important implications on electrical properties of resulting material under progressive ripening. The correlation between parameters was evaluated as the following.

Physico-chemical characterization

Physical properties of *Papayas* during ripening process are summarized in Table 2. It is worth mentioning that each letter in the side of corresponding value reveals the parameter level in the overall range of variation and the degree of statistical significance in assays. Averages with the same letter do not differ statistically from each other. The Tukey test was applied at a 5% probability level for averages considering quadruplicates.

As we can see, the TSS presents a positive variation as a function of time. According to Oliveira Junior et al. (2006) and Fonseca et al. (2003), a good indicator of ripening is established at a condition of high TSS (between 10.8 and 12.75%) and a slight decrease in AT (8.27 and 6.17 g kg^{-1} of citric acid in juice). If considered,

Table 2. Quantification of physico-chemical of stored *Papaya* (the variation coefficient was calculated from experiments in four different fruit per day)

Parameter	Days of storage					Variation coefficient
	0	2	4	6	8	
TSS (%)	8.025[c]	8.65[c]	9.9525 [b]	10.8[b]	12.75[a]	CV% = 4.10
TA (g kg^{-1})	8.95[a]	8.85 [a]	8.275 [b]	7.675[c]	6.175[d]	CV % = 2.47
TSS/TA	8.966e	9.774[d]	12.042[c]	14.047[b]	20.682[a]	CV % = 3.52
AI	2.34[a]	2.005 [b]	1.505 [c]	1.205[d]	0.605e	CV% = 8.20
LFW (%)	0e	7.085[d]	8.835 [c]	11.575[b]	14.29[a]	CV% = 6.71

TSS: Total soluble solids, TA: Titratable acidity, AI: Absorbance index, LFW: Loss of weight in fresh fruit.

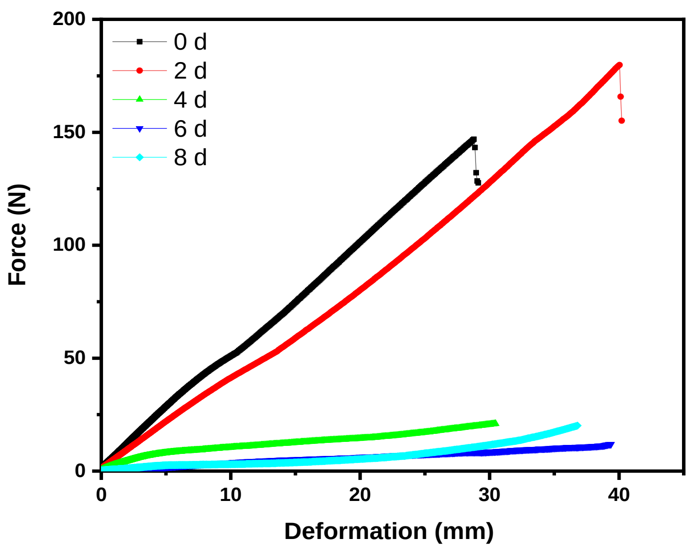

Figure 2. Dependence of force versus deformation in papayas at different post-harvest period.

the ratio TSS/AT makes it possible to verify a strong variation between the fourth and sixth day, in response of a combined increase of TSS and a decrease of AT. In the case of a climacteric fruit, it was observed that the maintenance of the fruit conservation is related to the quality parameters.

On the eighth day, extreme values were observed for fruit, with a higher TSS / AT ratio (20.682), a strong decrease in AT (6.175 g kg^{-1}, a TSS (12.75%) and an absorbance index (0.605), indicating that the fruit were already entering to senescence phase. Similar results were found by Oliveira Junior et al. (2006) with *Papaya* 'Sunrise alone'. It was observed an increase in the fresh mass loss during storage and according to Santos et al. (2008) a loss over 5 % of it is sufficient to depreciate *Papaya* fruit. In this work, that level (5%) of LFW occurred after 2 d of harvest, presenting a considerable statistical growth during storage, corroborating with the corresponding level described by Dias et al. (2011).

Mechanical assays

The mechanical resistance of *Papaya* at different ripening stages introduces important information related to the quality of fruit after harvest. One of the most significant parameters for quality of *Papaya* for consumers is its firmness since it reflects the ripening stages of the fruit (Jha et al., 2010).

Results of mechanical assays performed with fruit at different ripening degree (0, 2, 4, 6 and 8 d after harvest) are summarized in Figure 2. As we can see in Figure 2, a strong force is required to provide a lower level of deformation in fruit at first day. The complete epicarp disruption is reached for a force in the order of 150 N and a corresponding deformation of 30 mm characterizing the firmness of fruit. With progressive ripening, it is observed that is possible to increase the maximum force applied on the fruit surface, due to an increase in the maximum deformation, the elasticity of fruit increases with the ripening process. The elasticity is the capacity of a material for taking elastic or recoverable deformation. In this sense, this variation observed in elasticity as the fruit matures indicates that the resistance to bruising and damage is slightly lowered. In the following step of maturation, specifically at the third day but before the fourth after harvest, a remarked variation takes place in the mechanical properties of fruit (in agreement with previous data, about physico-chemical characterization) indicating that an accelerated depreciation of fruit takes place during this period.

After the fourth day, a minimal variation in force and deformation is observed, due to senescence and severe depreciation of fruit as it is observed in the low variation of mechanical resistance during the following days of ripening. That fact reduces the potential use of the mechanical assays for a clear evaluation of the overall

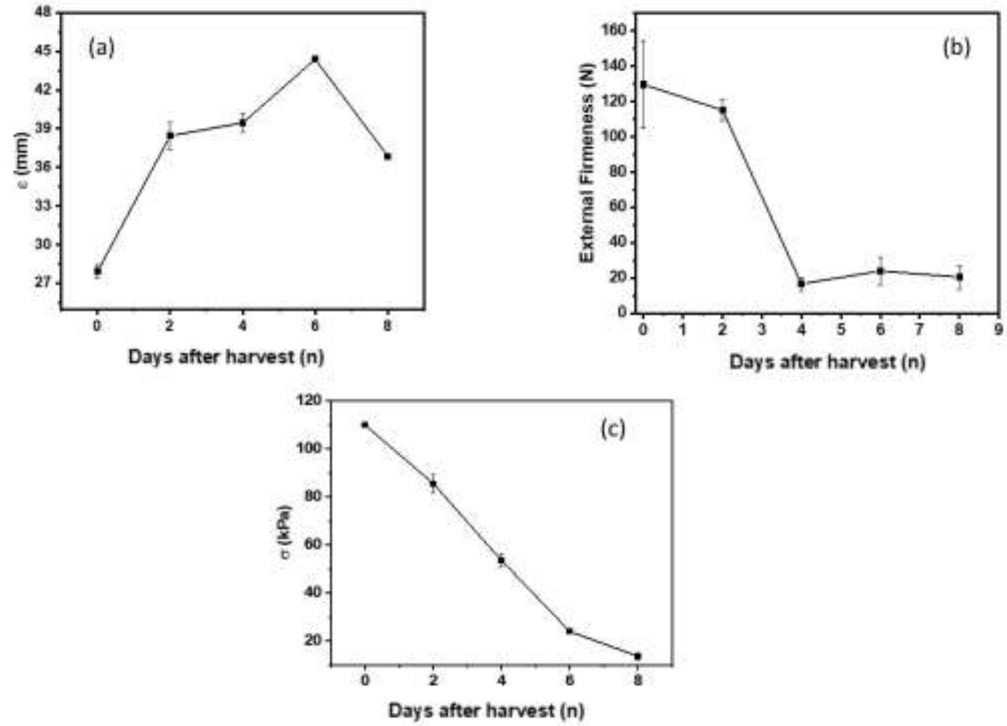

Figure 3. Mechanical assays of *Papayas* as a function of number of days after harvest: (a) external firmness, (b) deformation and (c) Henck tension. The error bar represents the deviation from medium value calculated from experiments in four different fruit per day.

kinetics of fruit degradation.

In order to evaluate the variation of different parameters in an independent manner with ripening stage, we plotted the behavior of firmness, elasticity and tension as a function of days of ripening (data shown in Figure 3). The external firmness of fruit (measured in N), shown in Figure 3a, presents a general decreasing trend in its value with strong variation between 2 d and 4 d after harvest, characterizing a typical plateau in which high level of firmness is observed for fresh fruit (t<2 d of harvest) followed by a low level of firmness (in order of 20 N) for t> 4 d from harvest. The result confirms the variation observed in TSS (with statistical significance) in the period of time between 2 d and 4 d, as a consequence of a more accelerated process of ripening and depreciation of the fruit that can be clearly identified in mechanical resistance data.

In association to this typical behavior for firmness, the mechanical elasticity and the Henck tension are two additional parameters with relevant information about properties of fruit (under ripening and in response of mechanical efforts). The deformation (a different measurement of elasticity of the fruit as shown in Figure 3b) increases with ripening degree in response to the decreasing firmness of the fruit. This process reaches a maximum after six days of harvest in which severe degradation tends to affect resistance of fruit.

As a consequence, the Henck tension tends to be strongly reduced (see Figure 3c) in the initial days of ripening decreasing firmness and increasing contact area as a result of elasticity of fruit with a reduced variation at severe condition of ripening.

Electrical characterization

The electrical response of *Papayas* was characterized by electrical impedance spectroscopy technique from direct measurement of bulky properties of fruit at high frequency range. In this case, it is possible to measure not only the interfacial mechanisms of transport and polarization (strongly affected by interaction of electrodes and the fruit peel) but more relevant bulky parameters - as previously reported for corresponding experimental systems (Figueiredo Neto et al., 2017). The mechanisms of ripening stage in *Papayas* is a function correlated to TSS and TA level which affects the transport mechanisms of current circulation in samples, as they are associated with the water content. As a consequence, the real part of impedance measured at high frequency range (1 MHz) was established as the most adequate parameter to characterize the bulky properties of

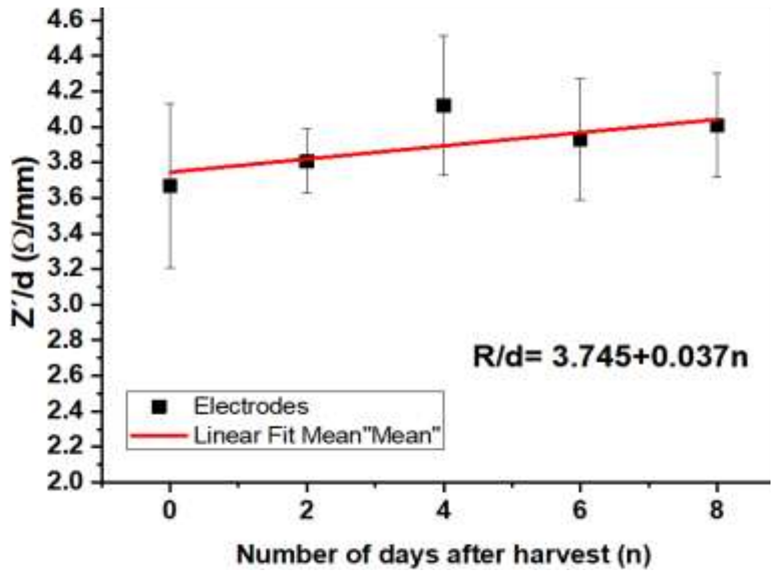

Figure 4. Dependence of real part of impedance of papaya (at 1 MHz) as a function of ripening degree. The error bar represents the deviation from medium value calculated from experiments in four different fruit per day.

transport mechanisms in *Papaya* since it helps to develop a single-point and non-invasive technique for identification of its ripening degree, minimal variation was observed in other range of frequencies due to variation in interfaces with degradation process.

Other important aspect to be addressed refers to the influence of the distance between electrodes on overall response of impedance. If considered the typical variation in the size of fruit, it is relevant to normalize the bulk resistance by diameter of fruit in order to evaluate a more intrinsic property from an electrical measurement.

Results in Figure 4 indicate a general trend, in which the normalized impedance increases with the days after harvesting of the *Papaya*. In spite of the low slope in the general behavior, an important aspect can be observed from this data, a small deviation of impedance at the fourth day of ripening. Despite the small displacement of curve from general slope (first order curve) it represents an elevation in normalized resistance that is in agreement with variation observed in corresponding parameters. Contrary to the mechanical data, the structural modification in the fourth day not affects the electrical property of material and initial variation that can be explored in the measurement of the ripening stage at severe condition.

If considered a more complex function to describe this data (than a simple first order function), it is possible to identify a stronger variation in the slope of curve that is in agreement with the corresponding variation in the firmness. Thus, this function can be considered as an important aspect in the kinetics of ripening.

The correlation between pairs of parameters explored from physico-chemical, mechanical and electrical properties were performed according to the first derivative of each parameter as a function of the number of days of ripening. The direct measurement of the slope variation per day can be explored in the determination of the overall kinetics of ripening. Figure 5 presents the variation of the slope for TSS (d(TSS)/dn) and the negative for TA (-d(TA)/dn): the signal is associated to the inverse variation of both parameters.

As we can see, the slope increases in the modulus of both parameters with the time, characterizing a strong variation in TSS and TA. An important aspect to be considered for the first derivative of the TSS refers to the inflexion point in the period between 2 d and 4 d, in which the slope remains with minimal variation.

Figure 6 shows the first derivative of firmness (dF/dn) and the negative of impedance (Z´/d) – d(Z/d)/dn as a function of time of harvest. The negative in derivative of impedance was established due to the reverse variation of both parameters contributing to the clearer visualization of both curves in the same plot.

As we can see, the first derivative of firmness with ripening (dF/dn) presents a strong variation in its value (the slope) at day two, in agreement with the corresponding value of firmness, strong reduction between 2 d and 4 d. After two days of ripening, the slope tends to lower values (plateau in the lower firmness values, severe ripening degree). In correspondence, the plot of -d(Z/d)/dn presents a similar variation with increasing the number of days after harvest as observed

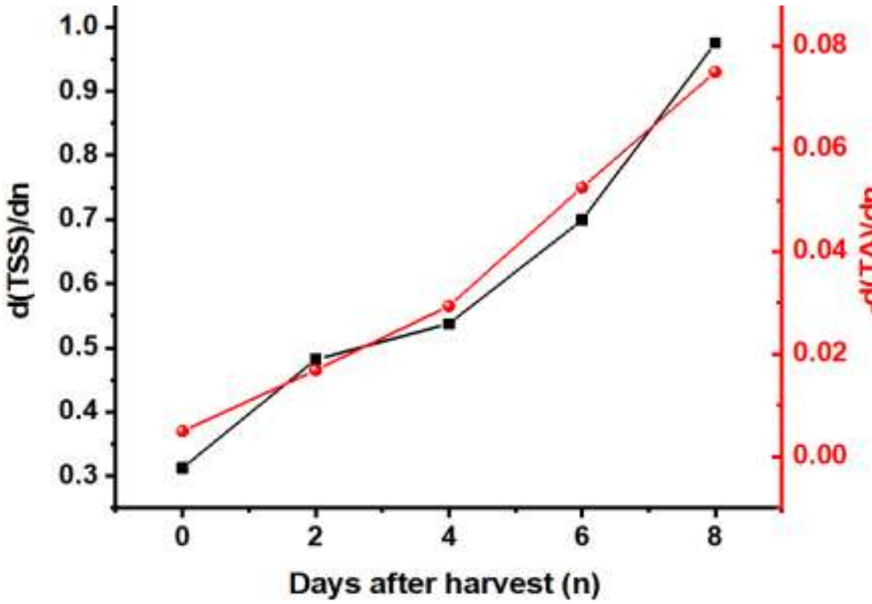

Figure 5. Comparison of first derivative of TSS and TA as a function of ripening degree.

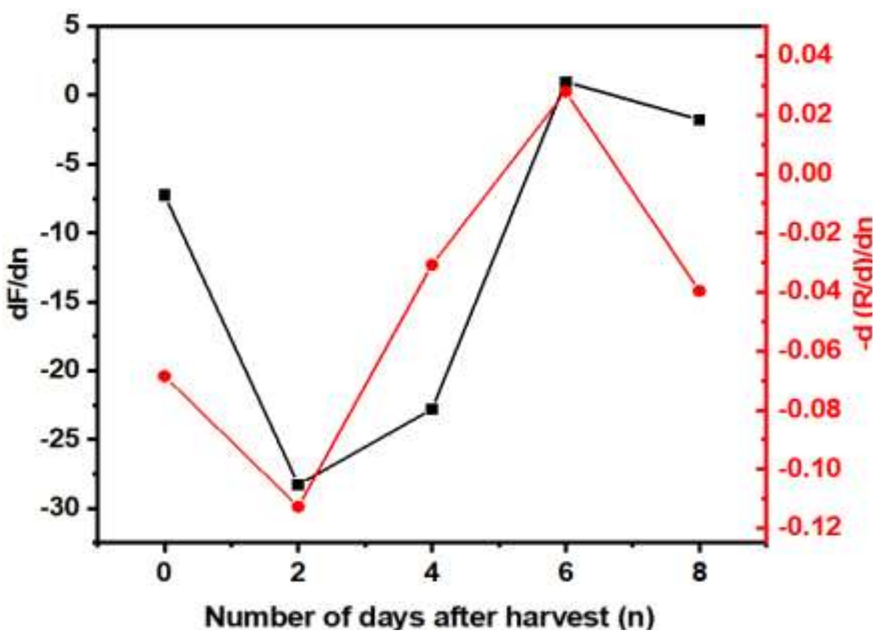

Figure 6. Comparison of first derivative of firmness and impedance as a function of ripening degree.

in the linear fitting, the medium in slope is in the order of 0.037 in the plot is possible to identify that at day two the slope presents a significant shift to a value in the order of 0.10. The correlation between these behaviors comparison of destructive (mechanical assay) and a non-invasive method (electrical impedance) confirms that the overall ripening process in terms of a detailed kinetics can be addressed by both techniques with the advantages of a non-invasive technique (the electrical impedance spectroscopy) that preserves the fruit and provides information relative to the degree of ripening and transitions detected by destructive assays beyond the limit of severe senescence of *Papaya*.

These results confirm that the direct measurement of ripening stage of *Papayas* by electrical impedance spectroscopy returns a reasonable level of sensitivity towards the physiological changes of fruit tissues. Different variation in the slope of the real part of impedance presented a strong correlation between intrinsic physico-chemical and mechanical parameters of fruit, such as lower variation in TSS and reduction in firmness of fruit. As a consequence, the development of a portable simple circuit that measures the impedance at 1 MHz can be explored as an interesting tool for identifying the ripening stage in *Papayas*. If we consider a calibration curve (control experiments), it is possible to associate the measured impedance with ripening degree in non-destructive and simple assays.

Conclusion

The kinetics of ripening in *Papaya* was performed using destructive and non-invasive techniques. The comparison of results revealed that all of properties were strongly affected after the fourth day of storage, characterizing this period of post-harvest as a transition between fresh condition, high firmness, low values of TSS and high TA to more severe conditions of ripening in which a strong variation in TSS and TA were followed by a lower firmness confirming the degradation of fruit. The use of EIS as an alternative technique for measuring the ripening stage of fruit confirmed all of those characteristics and introduced important advantages related to the development of a single point measurement of electrical properties in nondestructive assays.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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