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## Full Length Research Paper

**Brown flax grown under different planting densities**

**Reginaldo Ferreira Santos<sup>1\*</sup>, Fabíola Tomassoni<sup>1</sup>, Douglas Bassegio<sup>1</sup>, Tiago Roque Benetoli da Silva<sup>2</sup>, Jair Antônio Cruz Siqueira<sup>1</sup>, Samuel Nelson Melegari de Souza<sup>1</sup>, Luiz Antônio Zanão Júnior<sup>1</sup> and Deonir Secco<sup>1</sup>**

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**This study aimed to evaluate the influence of the planting system and population densities in the culture of flax. The experiment was conducted on the campus of the State University of Paraná, in the year 2012, using the split-plot design, where the main plots were the planting systems (line and haul) and plots the densities (100, 150, 200 and 250 plants/m<sup>2</sup>). The characteristics evaluated were: plant height, fresh weight and dry weight of plant, number of capsules, fresh and dry mass of the capsules. Yield components of linseed showed a positive increase in the planting line, but did not fit the regressions tested. The increased density of plants/m<sup>2</sup> was detrimental when the crop was sown by broadcasting.**

**Key words:** *Linum usitatissimum* L., competition, population increase.

**INTRODUCTION**

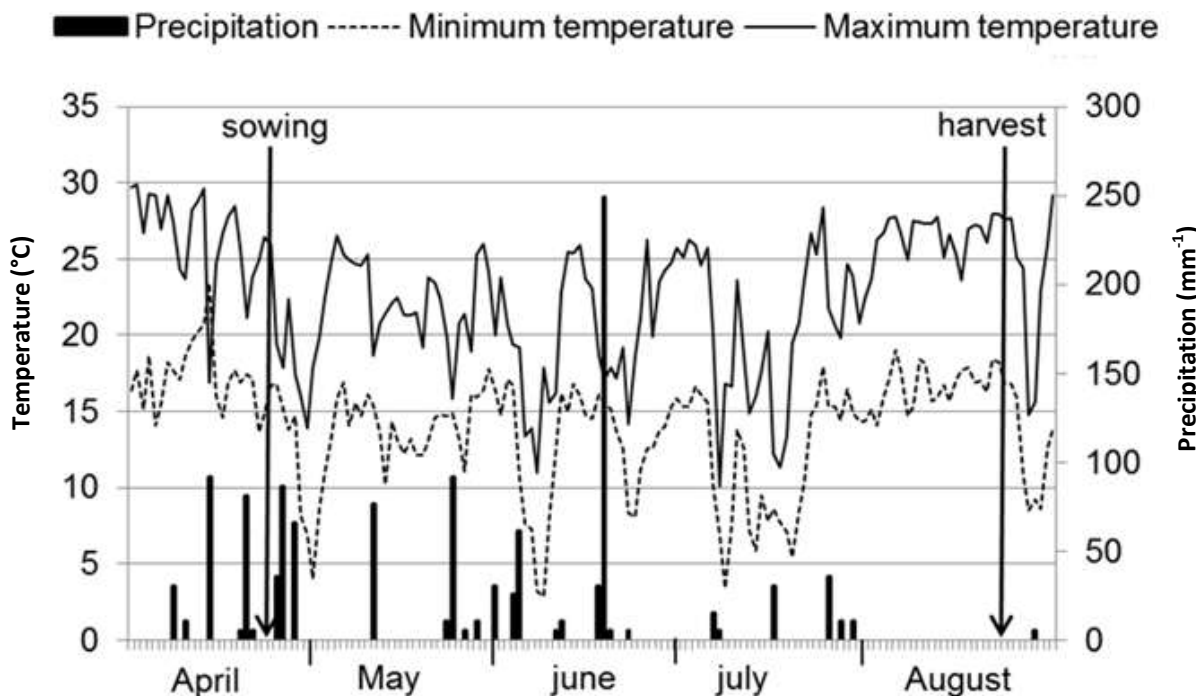
Originally from West Asia, flaxseed (*Linum usitatissimum* L.) had its benefits spread across continents and is commonly consumed in North America and in European countries (Bombo, 2006). Its seeds are rich in oil (about 40%), fiber (20 to 25%) and protein (20 to 25%), with a promising future in energy use in the production of biofuels (Rabetafika et al., 2011). It does not require great cultivation efforts; its cultivation is done many times in the process of crop rotation (Soares et al., 2009). According to Sattle (2000), several factors interact and influence the expression of the productive potential of flaxseed, among which stand out the process of sowing,

planting density and population.

These planting processes can lead to intraspecific competition (between plants) and interspecific competition (with other plants) for environmental resources such as light, water, nutrients, CO<sub>2</sub>, among others, causing damages in growth, development, and consequently the production of crops (Zanine and Santos, 2004). These competitions happen due to elevated seedling vigor, leaf expansion, formation of dense canopy, plant height, long development cycle and rapid growth of the root system (Sanderson and Elwinger, 2002).

Brendolan et al. (2000) evaluated the effect of mineral

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**Figure 1.** Precipitation (mm<sup>-1</sup>) and temperature (°C) recorded during the experiment with the culture of flaxseed.

nutrition on the competition between *Eucalyptus glandis* and *Brachiaria decumbens*, and observed that the intraspecific competition for environment resources decreased on average 23% in root length, leaf area and both shoot and root dry matter of eucalyptus. Some species have greater competitive ability, quickly developing architecture to intercept light: rapid expansion of leaf area and rapid colonization of the upper layer of the canopy, favoring the growth and production of photosynthesis (Lemaire, 2001).

Studies have shown that flaxseed may respond differently to the density and spacing of plants. Several experiments demonstrated that the variation in plant population resulted in significant differences in yield. According to Khan and Bradshaw (1976), plasticity, which is the ability to respond to altered spacing, ensures the success of plant development, directly influencing the density.

An experiment was conducted at the campus of the State University of Western Paraná, in 2012, using the design of split plot, in which the main plots consisted planting systems (line and haul), and the subplots by densities (100, 150, 200 and 250 plants / m<sup>2</sup>), Tomassoni et al. (2013) found the plant height behavior, fresh and dry mass of the plant, number of capsules, fresh and dry mass of the capsules. According to the authors, linseed production components showed positive growth in the sowing haul, but did not fit tested regression and increased plant density / m<sup>2</sup> was detrimental when the crop was sown in the line.

Diepenbrock and Pörksen (1992) found maximum seed yields with lower population densities, 200 and 400 plants/m<sup>2</sup>, respectively. Lisson and Mendham (2000) observed that the increase in population from 390 to 530 seeds / m<sup>2</sup> provides increased performance. Turner (1991) noted that the number of capsules doubled when the population went from from 400 to 900 seeds/m<sup>2</sup>.

However, with the enormous edaphoclimatic diversity and lack of studies on flaxseed, as well as the population density of the crop, the present study aimed to evaluate different population rates on plant development.

## MATERIALS AND METHODS

The work was conducted in the experimental field of Western Paraná State University (UNIOESTE), located in Cascavel, Paraná, Brazil, at latitude 24°53'47" S and longitude 53°32'09" W. The average annual rainfall is 1,640 mm and the average temperature is 19°C. The soil is classified as typical Haplorthox, with clayey to very clayey texture, undulated relief and basalt substrate (EMBRAPA, 2006). The climate is temperate mesothermal and super humid, climate type: Cfa (Koeppen) (IAPAR, 2011). The average monthly temperature and precipitation are shown in Figure 1.

The experimental design consisted of a split-plot arrange. The main plots were composed of two planting systems (line and broadcast), and subplots consisted of four densities: 100, 150, 200 and 250 plants/m<sup>2</sup>, with four replications.

The sowing of brown flaxseed was held manually on 10 April, 2012, in conventional tillage system. Halfway through the main plot, a spacing of 0.36 m was used for line planting. Base fertilization and crop processing was not performed throughout the experiment. Each plot measured 5 m wide and 5 m long, constituting 25 m<sup>2</sup>.



**Table 1.** Analysis of variance for height, number of capsules (N/C), fresh weight of plant (FWP), dry weight of plant (DWP), fresh weight of capsules (FWC) and dry weight of capsules (DWC).

Treatment	Height	N/C	FWP (g)	DWP (g)	FWC (g)	DWC (g)
Line	67.50	25.18 <sup>a</sup>	4.50 <sup>a</sup>	1.88 <sup>a</sup>	2.06 <sup>a</sup>	0.80 <sup>a</sup>
Haul	64.56	9.50 <sup>b</sup>	1.73 <sup>b</sup>	0.81 <sup>b</sup>	0.81 <sup>b</sup>	0.25 <sup>b</sup>
CV(%)	6.32	25.54	22.56	22.22	23.27	17.23
Test values F						
S.P.	3.96 <sup>n.s</sup>	100.37 <sup>**</sup>	124.00 <sup>**</sup>	102.57 <sup>**</sup>	112.60 <sup>**</sup>	289.05 <sup>**</sup>
Density						
CV(%)	8.60	35.58	38.74	35.01	33.26	44.46
I (SxD)	1.60 <sup>n.s</sup>	0.48 <sup>n.s</sup>	0.62 <sup>n.s</sup>	0.59 <sup>n.s</sup>	1.29 <sup>n.s</sup>	0.09 <sup>n.s</sup>

Means with different small letters in the columns are statistically different at (\*\*) 1% and (\*) 5% of probability or no significant (<sup>n.s</sup>) Tukey test.

During harvest, at 140 days after sowing, the following characteristics were evaluated: plant height (with the aid of a measuring tape), number of capsules per plant, capsule fresh and dry matter, plant fresh and dry matter (measured on a precision scale). Dry matter was determined after the samples were kept in a greenhouse at 60°C + -5°C, within 7 days, when there was no difference in dry matter in the period of 24 h.

The results were submitted to analysis of variance and their means were compared by Tukey's test at 5% of probability, using the statistical package Assisat<sup>®</sup> version 7.5 beta (Silva and Azevedo, 2002). Doses were compared by means of regression analysis when significance was observed by the analysis of variance.

## RESULTS AND DISCUSSION

The crops that we grow for food need specific climatic conditions to show better performance in view of economic yield. Yield is dependent on edaphic and climatic factors. The response to population density is highly dependent on limiting factor(s) (Amin et al., 2015). One can observe in Table 1 that plant height was not influenced by the tillage system, as well as the arrangement, regardless of the tillage system. The sowing of flaxseed in lines did not adapt to the regressions tested, but showed higher means for yield components. The planting system and population density did not affect plant height (Figure 2A). Gabiana (2005) found contradictory results, by observing negative effect of population growth on plant height, with 52.3, 49.7, 48.9 and 47.5 cm for 238, 379, 583 and 769 plants/m<sup>2</sup>, respectively.

One may observe in Figure 2B that the number of capsules, and component which is responsible for crop productivity was significantly higher in the line planting system, but the increase in plants / m<sup>2</sup> influenced only the broadcast sowing system. Similar results were found by Gabiana (2005), who obtained 24.3 capsules/plant for 238 plants/m<sup>2</sup>.

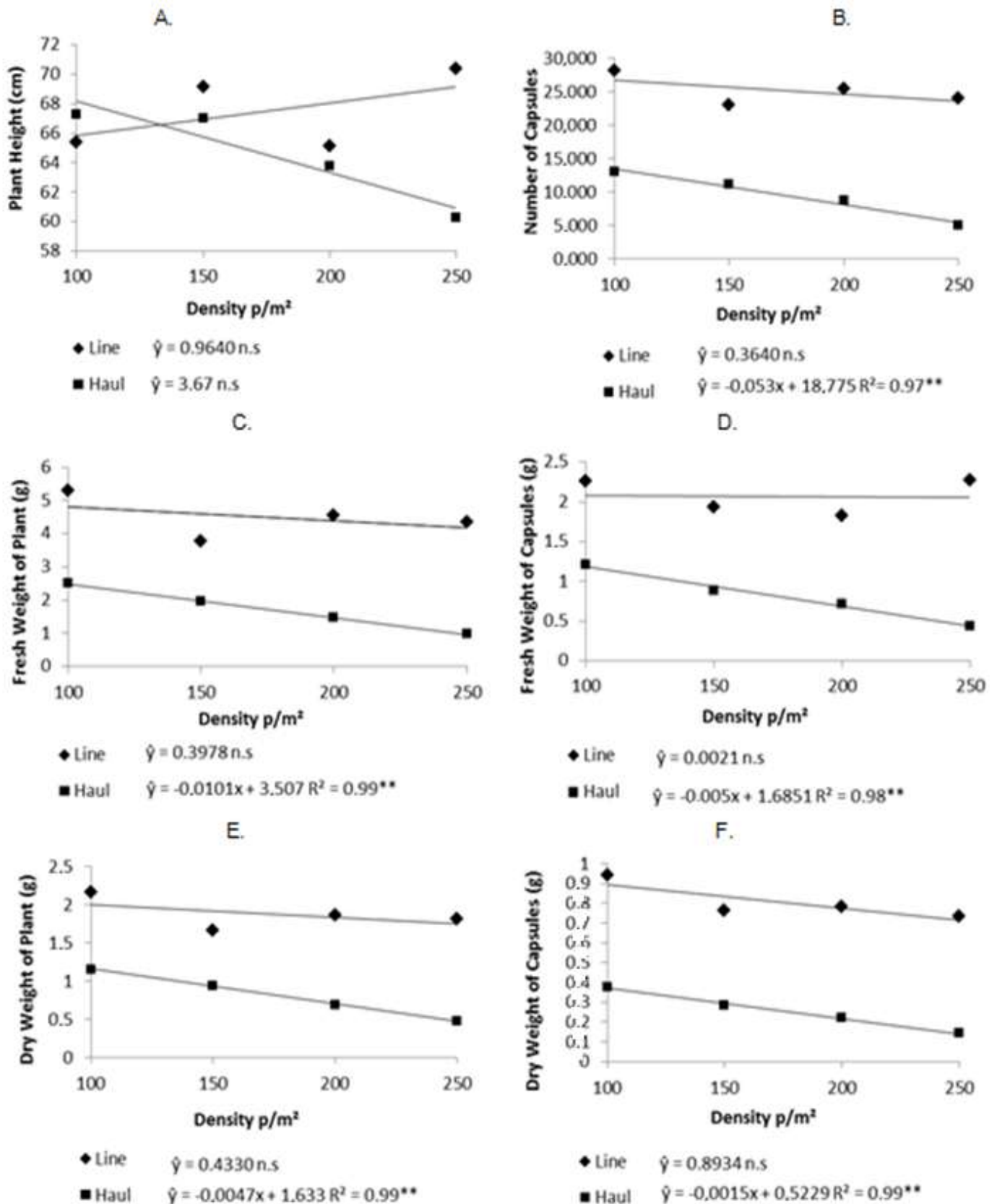
In the broadcast sowing system, the number of capsules per plant was adjusted in a negative linear way, showing the detrimental effect of plant density, corroborating partially with Gabiana (2005), who also observed a negative effect of population density increase in the variable, but for line sowing. Ceccon et al. (2004) observed that the lowest densities (60 and 120 plants / m<sup>2</sup>) provided higher number of panicles at the end of the oat crop. Fontoura and Moraes (2002) also studying an oat crop, observed effect of planting density, with higher grain yield for densities 200, 300 and 400 plants / m<sup>2</sup>.

Both fresh and dry matters of the plant (Figure 2C and E) were better explained according to the linear regression for the broadcast sowing system, however, the line sowing provided the best averages. Following the same trend of fresh and dry matter of the plant, the variable fresh and dry matter of capsules was higher in the line sowing system, even not being significantly adjusted. In the broadcast system, increased plant density/m<sup>2</sup> was detrimental to the accumulation of fresh and dry capsules. Casa et al. (1999) observed that flaxseed at low density had higher leaf area unity.

Bellé et al. (2012) in a work with the culture of safflower, found that the fresh matter of the stem was reduced when the culture was subjected to population growth. Gabiana (2005) observed no effect of the variation of plants per hectare for fresh matter of the flaxseed plant. Tomassoni et al. (2013) found that the golden flaxseed production components showed positive growth in online seeding adjusting to the tested regressions, and that increasing plant density / m<sup>2</sup> was detrimental when the crop was sown by broadcasting.

## Conclusion

Yield components of flaxseed showed a positive increase for the line sowing system, but did not fit the regressions



**Figure 2.** Plant height (A), number of capsules (B), fresh weight of plant (C), fresh weight of capsules (D), dry weight of plant (E) and dry weight of capsules (F). (\*\*) significant at 1% probability; (n.s) not significant.

tested. The increased density of plants/m<sup>2</sup> was detrimental when the crop was sown by broadcasting.

### Conflicts of interests

The authors have not declared any conflict of interest.

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## Full Length Research Paper

## Soil waterlogging during late season: Growth, assimilate partitioning and vigor of bean seeds

Tiago Pedó<sup>1\*</sup>, Geison Rodrigo Aisenberg<sup>1</sup>, Emanuela Garbin Martinazzo<sup>2</sup>, Velci Queiroz de Souza<sup>3</sup>, Carlos Eduardo da Silva Pedroso<sup>1</sup>, Tiago Zanatta Aumonde<sup>1</sup> and Francisco Amaral Villela<sup>1</sup>

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Late season crops in planossoil are prone to waterlogging associated with high temperatures that are characteristic of the season, during brief periods of time in early summer. The aim of the present study was to evaluate growth, assimilate partitioning and seed vigor of bean plants subjected to periods of waterlogging and high temperatures during late season. Bean plants of the IPR Tuiuiú genotype were submitted to conditions of soil field capacity and to waterlogging for 8, 16 and 24 h. In order to obtain growth data, plants were collected in regular intervals of seven days until the end of the crop cycle, starting after sowing, dry matter content and leaf area were determined and used to estimate dry matter production, relative growth and net assimilation rates, leaf area index, solar energy conversion efficiency and organs' dry matter partitioning. Seeds were collected at the end of the developmental cycle and used for seedling emergence test and evaluation of initial growth. The level of stress imposed by waterlogging and high temperatures is time dependent, paralyzing dry matter allocation in bean plants and reducing the conversion efficiency of solar energy. Seeds produced by plants under this stress present low vigor and reduced initial growth.

**Key words:** *Phaseolus vulgaris* L., stress, dry matter, physiological performance of seed.

### INTRODUCTION

Brazil, the world biggest common bean consumer, has Rio Grande do Sul as one of its states with highest per capita consumption. Common beans (*Phaseolus vulgaris* L.), belong to family Fabaceae, presenting an average lifecycle of 90 days (Ctsbf, 2010), at the Rio Grande do Sul State, the cultivated area of more than 3.3 million ha

with an average productivity of 1026 kg ha<sup>-1</sup> in the 2013/14 crop year (Conab, 2013). However, this state presents major climatic fluctuations, especially in relation with the quantity and distribution of pluvial precipitation. In such cases, sowing date, set according to the crops' agro-ecological zoning, is anticipated or delayed by the

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farmers (Ribeiro et al., 2008; Conab, 2013).

The stress imposed by soil water saturation causes morpho-anatomical and physiological alterations in plants. In those conditions, maize plants tend to reduce the number of leaves, dry matter accumulation (Coelho et al., 2013) and leaf expansion, and to induce leaf abscission (Bailey-Serres and Voesenek, 2008). Yet, in common bean plants there is an imbalance on the production and distribution of photoassimilates, due to an decrease in the conversion efficiency of solar energy (Didonet and Silva, 2004). In common beans, the excess of water during flowering may produce losses up to 60% in seed production (Silva et al., 2006).

In flooded soil regions, gas diffusion is hampered (Jackson and Colmer, 2005) reducing oxygen levels (Fries et al., 2007). Those conditions cause the diversion from the aerobic to anaerobic metabolism which reflects on low energy yields (Kolb and Loly, 2009); this metabolic modification induces the increase in the synthesis of enzymes that utilize pyruvate as the substrate to produce lactate and ethanol (Shingaki-Wells et al., 2011) leading to prejudice on root growth and metabolism (Amarante et al., 2007) and reducing nutrient absorption by roots (Pires et al., 2002), negatively affecting morphology and growth of various species.

High air temperatures also represent a limiting factor for commercial exploitation of beans (Junior et al., 2007). Consecutive periods under high temperatures may lead to damage on crop development (Barbano et al., 2001), chiefly on flowering and fructification, being a decisive factor on production due to its influence on flower abortion and pod formation (Didonet, 2002).

Late-season crops, in January and February, have shown growth trend on small farms, seeking for higher incomes. However, cultivations performed during this period are prone to soil waterlogging during brief periods of time, water saturation due to high pluvial precipitation, primarily on regions of planosol with a B textural horizon (Streck et al., 2008). Furthermore, high temperatures, common of the season, may negatively affect the crop and the performance of vegetal growth, which is a major consideration for high-quality seed production.

Given the aforementioned, the work intends to analyze growth, assimilate partitioning and vigor of seeds from common bean plants subjected to periods of soil waterlogging in late-season cultivation.

## MATERIALS AND METHODS

The experiment was performed in a chapel greenhouse, with a north-south orientation, in an experimental area of the Phytotechny Department at Universidade Federal de Pelotas – Capão do Leão, RS. The region presents temperate climate with well distributed rainfall and hot summer, Cfa type by Köppen's climate classification. Bean seeds cv. IPR Tuiuiú were placed to germinate in black polyethylene pots with 12 L capacity containing substrate soil collected from an A1 horizon characterized as a Haplic Eutrophic Solodic Planosol, previously amended according to soil

analysis and based on the "Fertilization and liming manual for the states of Rio Grande do Sul and Santa Catarina" (Cqfs RS/SC, 2004).

The plants were cultivated from January to March, 2014, which is the late-season for this crop. According to the climate normal for the period of 1971 to 2000, the mean values of maximum air temperature are 28.2, 27.9 and 26.9°C for January, February and March, respectively (latest data for the region). During the course of the experiment, the mean maximum temperatures were 30.5, 29 and 26.2°C for the respective aforementioned months. Yet, temperature data obtained by a thermometer located inside the greenhouse indicate mean maximum temperature values of 39°C during the period of the evaluations.

In the V4 growth stage (Ctsbf, 2010) the periods of soil waterlogging were applied, corresponding to eight, 16 and 24 h of waterlogging. An additional treatment consisted of the maintenance of soil field capacity, which was determined using tension table equipment (Embrapa, 1997). The waterlogging was performed providing a water level of 20 mm aboveground by fitting each pot inside a second pot with no perforations, preventing soil aeration and gas exchange. For drainage, these external pots were later removed allowing drainage until the field capacity.

In order to obtain primary growth data of leaf area and dry matter content, successive harvests were performed according to a seven day regular intervals alongside the whole crop cycle. In every collection, plants were cut at ground level, split into plant parts (leaf, shoot, roots and pods, if present) and accommodated separately kraft paper envelopes. Dry matter was obtained using four repetitions during each collection time and treatment; the samples were later transferred to a force ventilation oven, at the temperature of 70±2°C for 72 h.

The leaf area ( $A_L$ ) was determined using the equipment Licor model LI-3100 and leaf area index (LAI) calculated by the formula:  $LAI = A_L/S_t$ , where  $S_t$  corresponds to the superficial area occupied by the plant. Primary data of total dry matter accumulated ( $W_t$ ) were adjusted by the simple logistic equation,  $W_t = W_m/(1 + Ae^{-Bt})$ , where  $W_m$  stands for maximum growth asymptotic estimate,  $A$  and  $B$  represent the constants,  $e$  is the base of the Napierian logarithm and  $t$  the time in days after transplant. Leaf area primary data were adjusted using orthogonal polynomials (Richards, 1969). The instantaneous value for the rate of dry matter production ( $C_t$ ) was obtained from the derivative of the fitted equation of total dry matter (Radford, 1967). In order to obtain the instantaneous values for relative growth ( $R_w$ ) and net assimilatory ( $E_a$ ) rates, the equations  $R_w = 1/W_t \cdot dW_t/dt$  and  $E_a = 1/A_t \cdot dW_t/dt$  were employed, accordingly to Radford (1967).

Conversion efficiency of solar energy ( $\xi$ ) was determined from the equation  $\xi = (100 \cdot C_t \cdot \delta) / R_a$ , where  $R_a$  is the mean value of incident radiation ( $\text{cal m}^{-2} \text{dia}^{-1}$ ) fourteen days before the corresponding  $C_t$ , registered using a pyrometer and  $\delta$  is the calorific value of 3800  $\text{cal g}^{-1}$  cited by Cuesta et al. (1995). Assimilate partitioning between different plant organs (root, shoot, leaves and pods) along plant development were determined, separately, through measurement of the mass allocated in each plant structure followed by data transformation to percentage.

At the end of the crop cycle, seed harvest was performed for use in seedling emergence test which was accomplished using eight subsamples of 50 seeds each treatment. Every repetition was disposed to germinate in black polyethylene trays filled with the soil previously detailed and held at soil field capacity at the greenhouse. The following variables were assessed:

Seedling emergence at 21 days from sowing date  
Emergence speed index, obtained by daily count of emerged seedlings, accordingly to Nakagawa (1994).

At the end of the seedling emergence test, leaf area and dry matter of leaf, shoot and root were evaluated by measurement of 10

seedlings each sample. The experimental design was completely randomized with three repetitions, factorial scheme 4 x 10 (waterlogging period and harvest times), performing weekly randomization. Data concerning seedling emergence, emergence speed index, leaf area and organ's dry matter were subjected for analysis of variance and, when F-statistic was significant, Tukey-test was applied at 5% probability level. Primary data of total dry matter, leaf area and dry matter of leaves, roots, shoot and pods were subjected to analysis of variance, wherein data referring to growth analysis, were submitted to the simple logistic regression (Radford, 1967).

## RESULTS AND DISCUSSION

Total dry matter production ( $W_t$ ) of common bean plants at different waterlogging periods fitted the logistic tendency with a coefficient of determination higher than 80% (Figure 1a). The growth was slow until 21 days after sowing (DAS) and presented maximum growth at the end of the crop cycle (70 DAS).

Plants cultivated under field capacity conditions or 8 h of waterlogging showed increasing growth up to the 70 DAS. However, maximum dry matter accumulation occurred at the 42 DAS for plants subjected to 16 and 24 h of soil waterlogging, presenting loss of vitality after this period. The difference in the allocation of  $W_t$  on plants exposed to periods of 16 and 24 h comparatively to other periods can be related to the high temperatures that occurred during the crop cycle, leading to stoppage of growth and premature plant death (Figure 1a). Temperature values above 27°C are harmful to plants under the effect of soil waterlogging (Lizaso et al., 2001). Maximum dry matter production rate ( $C_t$ ) was achieved at 42, 63, 27 and 28 DAS in plants maintained at field capacity and under waterlogging for 8, 16 and 24 h, respectively (Figure 1b). Plants subjected 8 h of waterlogging presented a higher  $C_t$ , corroborating with the higher  $W_t$  (Figure 1c) and demonstrating that plants under waterlogging reduce the metabolism during and after the stress, extending crop cycle.

Relative growth rate ( $R_w$ ) presented maximum values at early development (seven DAS) with later systematic decrease until the end of common bean crop cycle (Figure 1c). The high initial  $R_w$  can be attributed to a high photosynthetic capacity of young leaves and later decrease, due to auto-shadowing, caused by the development of new leaves (Lopes et al., 1986).

Throughout the cycle, greater values of  $R_w$  were found in plants subjected to 8 h of waterlogging, followed by plants kept at field capacity (Figure 1c), possibly due to the occurrence of soil waterlogging causing a greater stagnation of dry matter increment relative to the preexisting. However, in plants under longer periods of soil waterlogging (16 and 14 h),  $R_w$  values decreased quickly and prematurely. Even though different genotypes present different responses regarding  $R_w$ , it is worth mentioning that under temporary waterlogging, three varieties and

four accessions of *Panicum maximum* Jacq. Presented lower relative growth rates (Silva et al., 2009).

Leaf area index (LAI) presented high coefficient of determination ( $R^2 \geq 0.97$ ) and, at 63 and 70 DAS, the maximum value was reached in plants maintained at soil field capacity and under the effect of 8 h of soil waterlogging, respectively (Figure 1d). Nevertheless, plants that had 16 or 24 h of soil waterlogging, the maximum LAI was verified at 28 DAS (Figure 1d). The decrease, after the peak, is the result of an increase on leaf senescence and alterations of hormonal balance (Moura et al., 2008).

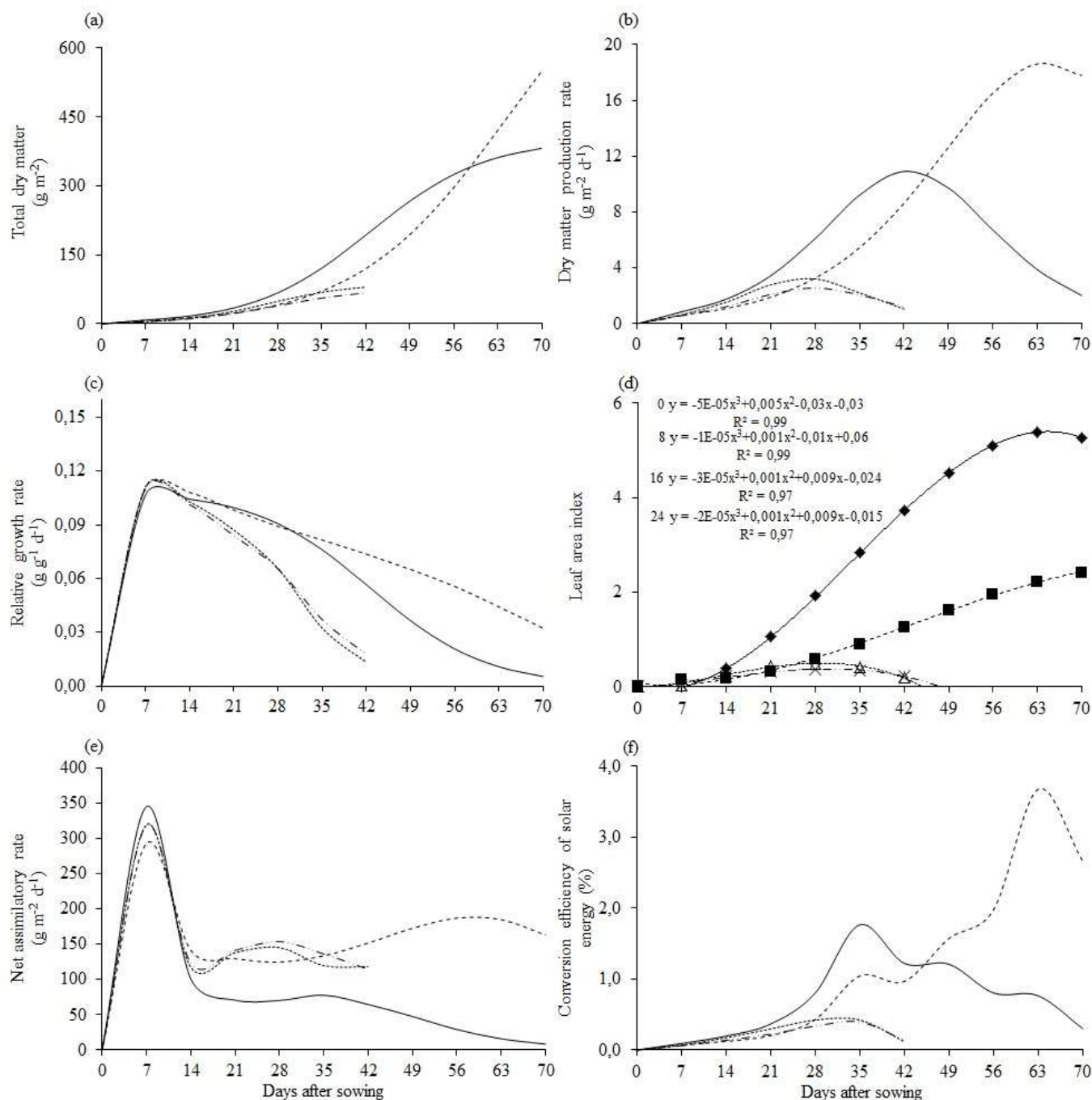
The maximum values of net assimilatory rate ( $E_a$ ) were obtained at early vegetative growth (7 DAS) with secondary peaks at 35 and 56 DAS for plants maintained at field capacity, and under the effect of 8 h of waterlogging, respectively (Figure 1e). At the early development,  $E_a$  tends to be higher (Gondim et al., 2008), the later decrease is associated with auto-shadowing and the second peak, results from the initiation of the reproductive stage (Lopes et al., 1986).

The reduction of photosynthetic rate, stomatal conductance and water absorption by roots (Folzer et al., 2006) can result in low photoassimilate production at the leaves and consequent low translocation to other plant parts (Parent et al., 2008). Such aspects may have been affected by the combination of soil waterlogging and high temperature, leading to stoppage of carbon fixation and growth, followed by premature death of common bean plants.

The curves for conversion efficiency of solar energy ( $\xi$ ) presented different trends according to the treatment (Figure 2f). The maximum values of  $\xi$  were 1.75% for plants at field capacity, 0.43 and 0.40 % for plants subjected to 16 and 24 h of soil waterlogging, at the 35 DAS. However, for plants subjected to eight hours of soil waterlogging, the maximum  $\xi$  was 3.70% at the 63 DAS. Up to 42 DAS the maximum values were obtained at zero and 8 h of soil waterlogging, respectively. The  $\xi$  increased along with the maximum obtained for  $C_t$  (Figure 1d) and solar radiation (Figure 1b). Similar results were reported for soybean plants (Marenco and Lopes, 1998) and for common bean plants subjected to different nitrogen sources (Cuesta et al., 1995).

Dry matter partitioning was modified along the developmental stages of common bean plants (Figure 2). At field capacity, a greater dry matter allocation was observed on leaves up to the 42 DAS, followed by shoot and roots (Figure 2a). The accumulation of dry matter at pods started at 49 DAS, modifying preferential metabolic sink which reduced the allocation on leaves and roots. However, the period of 8 h of waterlogging extended the period of dry matter accumulation up to 49 DAS. After this period, accumulation on pods took place, resulting in diminution of accumulation on leaves and roots (Figure 2b).



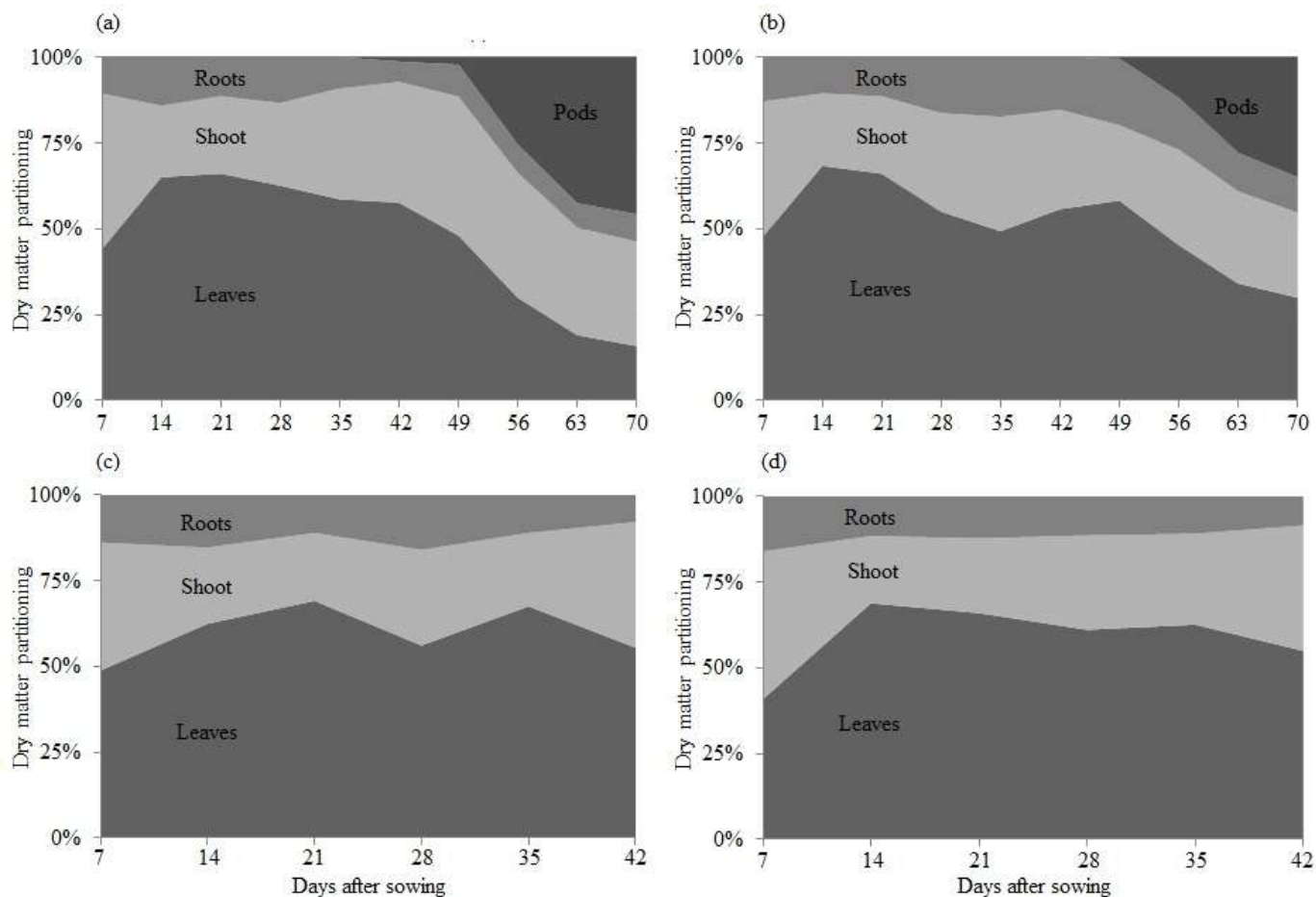


**Figure 1.** Total dry matter (a), dry matter production rate (b), relative growth rate (c), leaf area index (d), net assimilatory rate (e) conversion efficiency of solar energy (f) of common bean plants subjected to waterlogging periods during the vegetative stage. Plants maintained at field capacity (—), 8 (---), 16 (.....) e 24 (— · —) hours of soil waterlogging.

The responses of dry matter partitioning were similar on plants under 16 and 24 h of soil waterlogging (Figures 2c and 2d) ceasing the accumulation of dry matter at the 42 DAS, and without forming pods. Thus, the results obtained in this work evidence that extending the period of waterlogging associated with high temperatures leads to stoppage of growth and premature death on bean

plants.

The differences observed on dry matter allocation among plant organs, at the periods of zero and 8 h of waterlogging, can be associated with the fact that waterlogging reduces the production and translocation of photoassimilates from leaves to roots (Yordanova et al., 2004). Nevertheless, the absence of oxygen required to



**Figure 2.** Dry matter partitioning for different waterlogging periods on the vegetative stage for zero (a), eight (b), 16 (c) and 24 (d) h, on common bean plants.

**Table 1.** Seedling emergence (E), emergence speed index (ESI), leaf area ( $A_L$ ), and dry matter of leaves ( $W_L$ ), shoot ( $W_S$ ) and roots ( $W_R$ ) from seeds collected in plants subjected to field capacity and 8 h of waterlogging. UFPel, Pelotas, 2014.

Periods (h)	E (%)	ESI	$A_L$ (cm <sup>2</sup> )	$W_L$ (g)	$W_S$ (g)	$W_R$ (g)
Zero	94a <sup>1</sup>	7.57 <sup>a</sup>	43.25 <sup>a</sup>	0.12 <sup>a</sup>	0.05 <sup>a</sup>	0.08 <sup>a</sup>
Eight	88 <sup>b</sup>	6.09 <sup>b</sup>	35.37 <sup>b</sup>	0.09 <sup>b</sup>	0.04 <sup>b</sup>	0.05 <sup>b</sup>
CV (%)	2.35	10.39	4.06	5.49	4.78	8.31

<sup>1</sup>Means followed by the same letter are not different according to the Tukey-test ( $p < 5\%$ ).

reduce sugars by the glycolytic pathway, may have been a limiting factor for survival when periods of 16 and 24 h of waterlogging were used (Parent et al., 2008a).

The increase in the period of soil waterlogging tends to reduce dry matter accumulation of shoot and roots in plants of the Poaceae family, and the magnitude of the reduction is dependent of the genotype (Silva et al., 2009). Cultivation conditions under high air temperatures reduce the number of pods per plant, seeds per pod and

seed mass, which negatively affects the yield (Aidar et al., 2002).

The results of seedling emergence (E), emergence speed index (ESI), leaf area ( $A_L$ ), dry matter of leaves ( $W_L$ ), shoot ( $W_S$ ) and roots ( $W_R$ ) are presented on Table 1. Plants subjected to 16 and 24 h of waterlogging did not developed pods. Thus, the seedling emergence test, represented by the variables emergence and emergence speed index were performed solely using seeds originated

**Table 2.** Summary of the analysis of variance, mean squares for leaf area ( $A_L$ ), and dry matter of leaves ( $W_L$ ), shoot ( $W_S$ ), roots ( $W_R$ ) and pods ( $W_P$ ).

Source of variation	DF	Mean square				
		$A_L$	$W_L$	$W_S$	$W_R$	$W_P$
Waa <sup>1</sup>	3	0.0121*** <sup>3</sup>	27793.68***	17348.58***	332.72 <sup>NS</sup>	13258***
HT <sup>2</sup>	9	0.0058***	5474.82***	3702.87***	1049.75***	11430***
Waa x HT	27	0.0016***	4678.23***	3773.56***	1032.26***	3947***
Error	78	0.0006	126.94	49.49	166.81	8.78
Total	119	-	-	-	-	-
Mean	-	0.037	45.12	34.51	17.88	17.92
CV(%)	-	62.62	24.97	20.38	72.25	16.53

<sup>1</sup>Waterlogging Periods; <sup>2</sup>Harvest times; <sup>3</sup>Level of significance (P = \*5%, \*\*1% and \*\*\*0.1%).

from plants subjected to field capacity or 8 h of soil waterlogging.

Seedling emergence was affected by soil waterlogging, in which the plant was maintained, resulting in seeds with a lower percentage of emerged seedlings and emergence speed index. The same was observed to leaf area and for dry matter of leaves ( $W_L$ ), shoot ( $W_S$ ) and roots ( $W_R$ ).

Lower values for emergence and emergence speed index demonstrate the decrease on vigor of common bean seeds collected in mother plants subjected to soil waterlogging. Since seed vigor is related to stresses occurred directly or indirectly on the seed, any stress during vegetative or reproductive stage affects seed quality. Therefore, vigor decrease is related to the environmental stress imposed, affecting the biosynthesis of storage compounds, the formation of cellular membranes and probably, enzymatic mechanisms involved in the hydrolysis and translocation of reserves from the cotyledons to the embryo, during the resumption of growth (Peske et al., 2012). This sense, the decrease of seed vigor, imposed by soil waterlogging, reflected in poor development of seedlings, reducing dry matter of shoot, roots and leaves and additionally, the leaf area.

A reduced leaf area imposes a smaller area for capturing radiant energy, which can affect the photosynthetic process due to reduction of absorbed radiation (Aumonde et al., 2011). Under conditions of soil hypoxia, the low energy production caused by photosynthetic reduction (Alaoui-Sossé et al., 2005) leads to the depletion of carbohydrate reserves and total protein synthesis (Parent et al., 2008).

The analysis of variance of primary data of growth is presented on Table 2. Analyzing the mean squares for leaf area and organs' dry matter, high level of significance (0.1%) between waterlogging conditions and harvest times was noticeable. Different aspects related to management, the use of water and soil have been studied with a view to maximizing the use of agricultural agro-ecosystems (Valipour, 2013), as irrigation efficiency (Valipour, 2014a; Yannopoulos et al., 2015) and drainage systems (Valipour, 2014b). Thus, soil waterlogging

causes low levels of oxygen in the roots, leading to fermentation with low energy production. In this work, it was found lower allocation of dry matter and reduced vigor of bean seeds produced under soil waterlogging.

Thereby, it was observed that growth and seed quality of common beans can be negatively affected according to environmental conditions. Thus, the selection of more propitious areas and proper management regarding cultivation conditions are paramount.

## CONCLUSION

The increase of soil waterlogging periods on late-season provokes alterations on growth and assimilates partitioning of common bean plants, delaying the attainment of the second peak of net assimilation rate. Soil waterlogging and high temperatures cause decrease in conversion efficiency of solar energy and seed vigor of the aforementioned specie.

## Conflict of interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Ethnopedology for solving problems of soil management and sustainable agriculture implementation in West Africa Savannah regions

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Recently many scientific studies have shown the relevance of ethnopedological informations in the different agro-ecological and socio-cultural context of Africa. However, information did not sufficiently serve in solving the problems of land degradation and famine affecting seriously Sub-Saharan nations. Strategies of best and wide use of indigenous knowledge are to be refined. The main objective of the present article is to analyze the ethnopedological informations and to discuss the possibilities of their integration to databases development. The structure and the quality of ethnopedological informations allow their integration in large and dynamic databases for better soil and environmental management and sustainable agriculture. Such databases must also include information from conventional academic studies (as biophysical, socio-economic and market studies).

**Key words:** Ethnopedology, savannah, West Africa, soil, sustainable agriculture, data base for development.

## INTRODUCTION

It is now widely recognized that traditional knowledge has been used during centuries for the rational management of natural resources, biodiversity and agro-ecosystems (Warren, 1992a; Barrera-Bassols and Zinck, 2003). Recently, many scientific studies, especially from the 1990s, have shown the relevance of local knowledge related to soils and land resources. Indigenous soil knowledge is very complex and include, among others, soil classification, farming skills and agronomic behavior of soils. This knowledge, in literature, has multiple qualifiers (traditional, local, native, indigenous ...) which are interchanged, but does not have the same etymological meaning. It refers, in all cases to ethnopedology. We must remember that the

ethnopedology, compared with ethnobotany and ethnozoology, is newly structuring (Barrera-Bassols, 2003), from the combination of natural and social sciences (soil science, geopedological inventory, social anthropology, rural geography, agronomy). Since 1989, the average scientific studies in ethnopedology is 33 per year (Barrera-Bassols and Zinck, 2003), resulting in a rapid growth of the discipline. In West Africa, particularly in the savannah regions, old and recent studies have been conducted in different agro-ecological and socio-cultural situations (Dabin, 1951; Warren, 1992b; Diallo and Keita, 1995; Diallo et al, 1998; Mikkelsen and Langohr, 2004; Yacubu et al., 2014). Despite this, local knowledge do not sufficiently serve as references in

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solving actual development problems. Generally, with the opening to the global economy, central governments seem to overlook local knowledge which are usually absent in development strategies. After decades of implementation of many rural development projects in this context, sub-Saharan African countries seem to have difficulties to limit land resources and environmental degradation and remain food insecure. Reversing this situation is a necessity that requires the implementation of strategies based on relevant information including those relating to soil and agricultural technologies. Such strategies can increase the chances of success in implementation of sustainable agriculture, better protection of soils, environment and biodiversity; they must use, in the case of soil resources, information from conventional agricultural research but also ethnopedology.

The main objective of this review article is to analyze the ethnopedological informations and to discuss the possibilities of their integration to databases for better soil and environmental management and sustainable agriculture.

## **ACCESSIBILITY TO RELEVANT INFORMATION FROM ETHNOPEDELOGY**

### **Difficulties**

Generally, scientists working on indigenous knowledge (IK) have often noted the difficulties of access to this knowledge (Gadgil et al., 1993; Diallo et al., 1998). These difficulties appear to be related to the complex nature of IK where concepts and practices are interconnected with local beliefs and religious practices. In some cases, access to such knowledge is hampered by the inadequacy of the methods used by modern science (Diallo et al., 1998). The difficulties do not always permit the identification of relevant information from local knowledge, those utilizable in dynamic databases for solving current development problems.

### **Research process for relevant information**

For better access to indigenous knowledge, each academic discipline of modern science must develop appropriate methods. A method applicable in ethnopedology was proposed in Mali (Diallo et al., 1998). It should include surveys of local perceptions of land, conventional field soil characterization, and soil samples analysis in laboratory. Previously, the physical context of the study must be defined relative to a known agro-ecological zone framework. People whose knowledge is sought by the study should be clearly identified in relation to linguistic and socio-economic frameworks well-

defined.

### ***Surveys of local perceptions of lands***

They must start with a consultation with representatives of the community, for example a village or group of villages. During a meeting, the researcher must be clear about the objectives of the study and create conditions for open collaboration. Subsequently, well-targeted surveys should cover a limited number of people (5 for example) but well recognized in the community as people particularly well informed on the soils and land resources. Questions should afford a list of soil types and characteristics that farmers attribute to them. This list, after the first few tentative inquiries, should be improved later.

### ***Conventional field soil characterization***

This characterization must be conducted with farmers. All soil types listed above must be identified in situ, using the criteria of the farmer and those of conventional pedology. The opening of graves should be performed at locations determined from the indications of farmers. The description of the environment of soil profile should particularly emphasize on topography, water regime, vegetation and land use practices. In the description of soil profile, attention must be granted to the characteristics which allow judging the abilities of agricultural land and anticipating problems arising from their cultivation. However, observations related to soil processes should not be neglected. Soil samples should be collected from representative profiles of soil types for laboratory analysis.

### ***Soil analyses in the laboratory***

Each soil type identified in a local system can be attached, without much difficulty to a well known scientific classification. The analyses will focus on some detail properties (particle size distribution, carbon, nitrogen, and pH, etc). Further analysis can be viewed in terms of financial and material resources and information already accumulated on the soil type.

### ***Summary of collected data***

The information provided by farmers and those obtained using the soil science methods (in situ and in laboratory) should be considered as a whole. Thus, it becomes possible to establish a diagnosis, define the average characteristics of each soil type of the studied system



**Table 1.** Indigenous hierarchical soil classification (case of Falo agroecological zone, Mali) in accordance with Diallo et al. (1998)

Soil type	Bèlè	Fuga (*)	Cincin (**)		Boa (**)		
			Cincin blé	Cincin fing	Boa blé	Boa fing	Boa diè
Topography		Cuirassed plateau	Upstream of the glaze	Downstream of the glaze		Alluvial terrace	Basin
Thickness and depth (cm) of horizons (H)	H1 =0-10 H2=10-40	H1=0-10 H2=10-30	H1=0-10 H2=10-40 H3=40-100	H1=0-20 H2= 20-90 H3= 90-120	H1= 0-10 H2= 10-40 H3 = 40-120	H1=0-10 H2=10-40 H3= 40-120	H1=0-20 H2=20-40 H3=40-120
Gravel content (%)	H1 =30 H2 = 60	-	-	-	-	-	-
Textural class	H1 : LS H2: SCL	H1 : SL H2: SCL	H1: S H2:LS H3:	H1:LS H2: SC H3:	H1:SL H2:SLC H3:	H1:SL H2:SC H3:	H1:SC H2:SC H3:
Soil color					H1:10YR4/4 H2:7.5YR6/6 H3:7.5YR8/6	H1:10YR4/3 H2:10YR6/3 H3:10YR8/3	H1:10YR5/2 H2:10YR6/2 H3:10YR7/1
Organic matter content (%)	H1= 0.71 H2 = 0.59	H1= 0.54 H2 =0.45	H1= 0.56 H2 = 0.46	H1= 0.31 H2 = 018	H1= 0.31 H2 = 018	H1= 0.99 H2=0.59	H1= 0.64 H2=0.41
Soil pH	H1= 5.28 H2 = 5.17	H1= 5.11 H2 =5.12	H1= 6.77 H2 = 6.80	H1=5.33 H2= 5.33		H1 = 5.91 H2= 5.46	H1= 6.30 H2= 6.38

Textural classes symbol signification: SL, Sandy loam; LS, loamy sand; CL, clay loam; C, clay; SCL, sandy clay loam; SC, sandy clay; Color symbol signification: 7.5 YR 8/6, reddish yellow; 10YR4/2, Dark greyish brown; 10 YR4/3, brown; 10YR4/4, dark yellowish brown; 10YR4/6, yellowish brown; 10YR5/2, grayish brown; 10YR5/6, yellowish brown; 10YR6/1, light gray; 10YR6/2, light brownish gray; 10YR6/3, light brownish gray; 10YR7/1, light gray; 10YR8/1, White; 10YR8/3, very pale brown

and establish equivalencies in other soil references (local and scientific ones).

#### ETHNOPEDOLOGICAL INFORMATION QUALITY

The quality of information from ethnopedology is an important factor when it is question to introduce this information in complex databases. Studies conducted by academic institutions,

particularly in Africa, permit today a pertinent analysis of soil information quality from ethnopedology.

#### Structure of soil classification in ethnopedology

The soil classifications studied in Mali (Dabin, 1951; Diallo and Keita, 1995; Diallo et al., 1998) are mainly from *Bambara* and *Malinke* ethnic

groups, which are in same dialectal entity (*Manding* dialect). *Manding* dialect is a spoken language in many countries of West Africa (Burkina Faso, Gambia, Guinea, Ivory Coast, Mali, Senegal, Sierra Leone). The criteria used to distinguish soils are the topographic position, the texture and gravel content and the color of the surface horizon.

Topographic criteria, often indirect, are particularly clear when it exists in the agro-ecological zone, reliefs showing a stark contrast

**Table 2.** Indigenous non hierarchical soil classification (case of Djitoumou, agro-ecological zone, Mali) in accordance with Diallo and Keita (1995).

Soil type	Fuga	Bèlè	Cincin	Bira	Fala
Topography	Cuirassed plateau	Cuirassed surface with moderate slope	Glaze	Basin	Depression along the river
Thickness and depth (cm) of Soil horizons (H)	H1= 0-10 H2= 10-25	H1=0-10 H2=10-50	H1=0-15 H2 =15-50 H3=50-140	H1=0-15 H2=15-55 H3=55-125	H1= 0-10 H2 =10-50 H3= 50-140
Gravel content (%)	H1= 25,3 H2=50	H1= 55,0 H2 = 61,0	-	-	-
Soil color	H1 :10 YR3/3 H2 : 10YR5/6	H1:10YR3/3 H2 : 10YR4/6	H1 : 10YR4/3 H2 : 10YR5/6 H3 :10YR6/4	H1 :10YR4/1 H2 : 10YR7/4 H3 :10YR7/2	H1 : 10YR5/2 H2 : 10YR7/2 H3 :10YR8/2
Organic matter content (%)	H1= 1.66 H2 = 0.79	H1= 1.98 H2 =1.33	H1= 0.90 H2 = 0.30	H1 = 1.23 H2 = 0.79	H1= 1.45 H2 = 0.71
Soil pH	H1 = 5.38 H2 =5.06	H1= 5.96 H2= 4.95	H1= 6.46 H2= 5.46	H3 = 5.92 H3 = 5.42	H1 = 5.31 H2 = 5.02

Textural classes symbol signification: SL, sandy loam; LS, loamy sand; CL, clay loam ; C, clay; SCL, L, sandy clay loam; L, loam.

Color symbol signification: 7.5 YR 8/6, reddish yellow ; 10YR4/2, Dark greyish brown; 10 YR4/3, Brown; 10YR4/4 , Dark yellowish brown; 10YR4/6, Yellowish brown; 10YR5/2 , Grayish brown; 10YR5/6 , Yellowish brown; 10YR6/1, Light gray; 10YR6/2 , Light brownish gray; 10YR6/3, Light brownish gray ; 10YR7/1, Light gray; 10YR8/1, White ;10YR8/3, very pale brown

to the rest of the space: Stony hills, cuirassed plateaus, large alluvial terraces and basins, colluvio-alluvial depressions along small streams. According topographic position, the main identified land categories are:

1. Kulu dugukolo on the stony hills;
2. Fuga on cuirassed plateaus
3. Lè dugukolo, linked to the alluvial terraces and basins;
4. Fala dugukolo linked to depressions along small streams.

Texture and gravel content criteria occur unambiguously in all peasant soil classifications and at higher level. It permits to distinguish three main types of soil: Gravelly soil (*Bèlè dugukolo*), sandy soil (*Cincin dugukolo*) and clay soil (*Bogo* or *Boi*).

Color, as the texture is a criterion always used, but at a secondary level. So, in textural categories, distinctions are still made according to color: black (*fiŋ*), red (*blé* ou *oulé*), and white (*diè*).

In some indigenous soil classification, soil type can be set from a particularly well displayed behavior. This is the case of *Dakissè dugukolo*, a soil type of a local classification in Kangaba region, Upper Niger basin in Mali. It is a subtype of *Lè dugukolo*, developed on an alluvial terrace of Niger River. Here, the term *dakissè* refers to the granular structure of the soil. In the local language, *dakissè* means the grain of *Hibiscus sabdariffa*.

Hierarchical classification (Table 1) is not always observed in all ethnopedology system, and classification can be limited to a repertory of soil types (Table 2). However each soil type has its attributes, enough clear without any confusion possibility.

The use, texture and color as criteria in indigenous soil classification has been noted by many other researchers working in West Africa (Warren, 1992b; Mikkelsen and Langohr, 2004; Yacubu et al., 2014) and other parts of the World (Barrera-Bassols and Zinck, 2003).

### Ethnopedological information related to soil qualities

In different agro-ecological situations, farmers know clearly the capabilities of every soil type of their classification system and also, the sensitivity of these soils to process as fertility decline under cultivation, runoff and erosion, etc. This observation appears in studies carried in Bagoé watershed (Sikasso region, Mali) as shown in Tables 3 and 4, according Kassogué (2013).

### Ethnopedological information and development of data base for soil management and sustainable agriculture

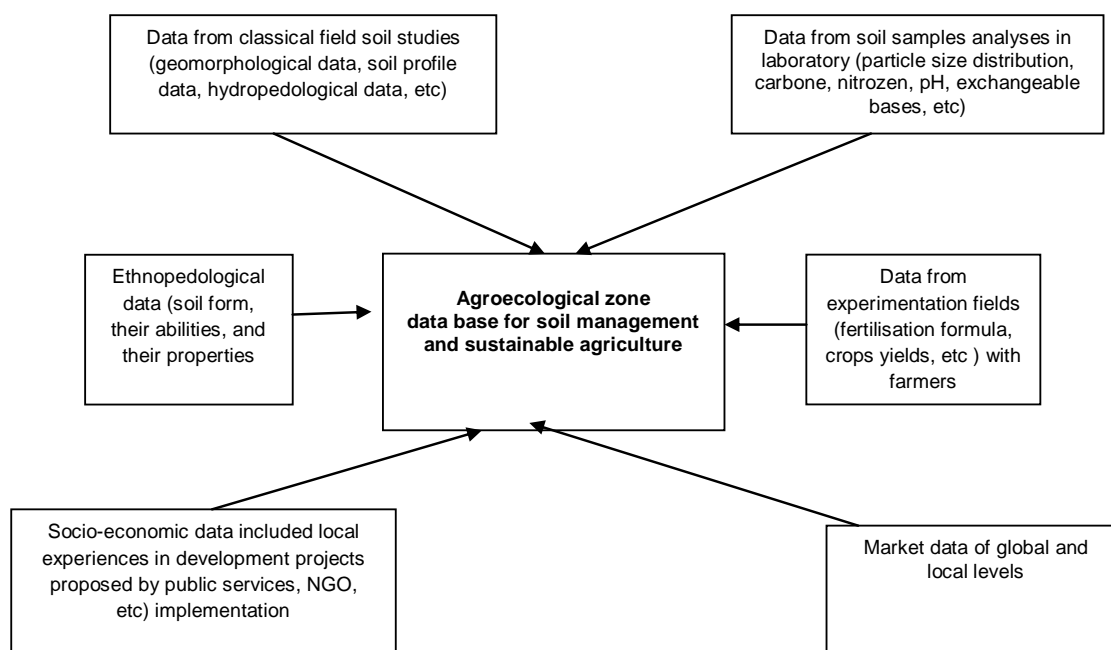
Ethnopedological information as examples describe above, are utilisable in data base for soil and

**Table 3.** Farmers appreciation of soils fertility decline in Bagoé basin (Sikasso region, Mali) in accordance with Kassogué (2013).

Rate of soil fertility decline	Classes		
	I: Very fast	II: Fast	III: Slow
Soil type	Nanga	Bogo , Cincin, Guini, koulou	Bèlè Fuga Mura

**Table 4.** Farmers appreciation of soils sensivity to runoff and erosion in Bagoé basin (Sikasso region, Mali) in accordance with Kassogué (2013)

Soil component	Classes			
	I : Very high	II : High	III : Moderate	IV: Low
Sensibility to runoff	Nanga	Cincin, Mura, Tiantian, Guini	Koulou, Fuga, Bogo, Bèlè	
Sensibility to erosion		Cincin, Muru, Guini,	Bèlè, Nanga, Koulou, Fuga, Tiantian	Bogo

**Figure 1.** Some categories of information needed for local data base for soil management and sustainable agriculture (adapted from Diallo, 2004).

environmental management and the implementation of sustainable agriculture. In this order, attention can be paid to local classification system, local people perceptions of each soil type (it is fundamental properties, abilities and sensitivity to major process, as the rate of fertility decline, proliferation of weeds, runoff and erosion). However, data from conventional academic studies are very useful and irreplaceable (Figure 1). But it is necessary to organize their acquisition and their use

with maximum attention. They must include data related to the biophysical and climate context, socio-economic characteristics, and market information both at global and local levels.

## CONCLUSION

The structure and the quality of ethnopedological

information allow their integration in large and dynamic databases for better soil and environmental management and sustainable agriculture. Such databases must also include information from conventional academic studies (as biophysical, socio-economic and market studies).

### Conflict of Interest

The authors have not declared any conflict of interest.

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## Full Length Research Paper

# Alternative methods of biological control in maintaining the viability of stored coffee seeds

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The objective of this work was to evaluate the efficiency of different alternative methods of biological control in maintaining the viability of coffee seeds. For the control of fungus contamination in seeds, dehydrated and powdered medicinal plants in doses of 200 g kg<sup>-1</sup> of seed, were tested with chemical fungicides mancozebe (Dithane® NT 4 g kg<sup>-1</sup> of seed), potassium sorbate (300 g L<sup>-1</sup>) and sodium benzoate (300 g L<sup>-1</sup>) and three biological products, Trichoderma® SP (1 g kg<sup>-1</sup> of seed), Trichodel® (50 g kg<sup>-1</sup> of seed) and Trichoplus® (50 g kg<sup>-1</sup> of seed). Samples of 200 g of seeds were conditioned in three different packages: polypropylene flasks, kraft paper bags and polyethylene + nylon bags. After, they were stored in cold chamber at 16 ± 3°C of temperature, for a period up to 15 months with evaluations for each three months. Seeds conditioned in kraft paper bags presented percentage of germination higher than those conditioned in polypropylene flasks or polyethylene + nylon bags. In seeds treated with rosmarin, garlic and Trichoplus®, there was a reduction in the total microbial, and the best germinated on 12 and 15 months of storage.

**Key words:** *Coffea arabica*, package, quality, storage.

## INTRODUCTION

Obtaining coffee seedlings with high quality standard and on time for cultivation is hampered by the non-conservation of the germinative power of seeds by periods higher than six months after harvest (SQUAREZI et al., 2001). During the storage, the viability of seeds is influenced by factors like species, cultivar, physiological quality, moisture content, relative air humidity, temperature, storage period, packaging type and the

action of fungus and insects (CARVALHO and NAKAGAWA, 2000).

Given the high incidence of microorganisms in important crops, the search for new antimicrobial agents extracted from plants, has been an alternative for the control of these pathogenic microorganisms, which is an alternative to the synthetic products. Beyond this, before now, there is no fungicide registered in the Agriculture

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Ministry Pecuary and Supplying (MAPA) to control microorganisms in coffee seeds during storage (MAPA, 2009).

Alternative methods that control proliferation of microorganisms in coffee seeds, without causing significant damages to the environment, have been intensely studied and the use of medicinal plants like alternative to the use of synthetic products is very promising (Souza et al., 2007).

Approaches developed with crude extracts and essential oils of medicinal plants have demonstrated the potential of natural products on phytopathogens (Moreira et al., 2004). The aim of this work was to evaluate the effect of medicinal plants, biological products and chemical alternative compounds on *Coffea arabica* seeds stored within different packages, up to 15 months in cold chamber.

## MATERIALS AND METHODS

The assays were developed in the following laboratories: Laboratory of Seeds for Research, Crop Science Department, Laboratory of Seed Pathology and Post-harvest of the Phytopathology Department, both at Federal University of Viçosa and Laboratory of Microbiological Analyzes of Food and Water of EPAMIG in Viçosa- MG, Brazil. The coffee seeds (*C. arabica* L.), cultivar Catuai Vermelho IAC 44 were obtained from the experimental Farm Vale do Piranga, Oratórios- MG Brazil, in 2012 and 2013. The fruits were manually selected in the cherry stage. Then, the fruits were peeled and desmucilled by natural fermentation per 12 h. After that, fruits were dried until the moisture content of 42% was achieved.

For the control of fungus contamination of seeds, the following dehydrated and powdered medicinal plants were tested: rosemary (*Rosmarinus officinalis* L.), basil (*Ocimum americanum* L.), garlic (*Allium sativum* L.), cinnamon (*Cinnamomum zeylanicum* L.), horsetail (*Equisetum arvense* L.), clove (*Caryophyllus L. aromaticus*), fennel (*Pimpinella anisum* L.), ginger (*Zingiber officinalis* W.) and basil (*Ocimum basilium* L.). The material was acquired from Flowers and herbal Pharmacist Ltda, Piracicaba-SP, and applied in doses of 200 g kg<sup>-1</sup> of seed. The chemical fungicide Mancozebe (Dithane® NT, Dow AgroSciences Industrial Ltda, São Paulo/SP - Brasil) 4 g kg<sup>-1</sup> of seed, potassium sorbate (300 g L<sup>-1</sup>) and sodium benzoate (300 g L<sup>-1</sup>) were also tested, and the seeds were immersed for one minute in each solution. At the end, three biological products were tested, Trichodermil® SP (*Trichoderma harzianum*, ESALQ 1306, Koppert Brazil, Piracicaba/SP – Brazil) 1 g kg<sup>-1</sup> of seeds, Trichodel® (*Trichoderma* spp., ECCB Caxias do Sul/RS - Brazil) 50 g kg<sup>-1</sup> of seeds and Trichoplus® (*Trichoderma* spp. and *T. harzianum* JCO Indústria e Comércio de Fertilizantes LTDA, Barreira/BA - Brazil) 50 g kg<sup>-1</sup> of seeds.

Samples of 200 g of seeds were stored in three different packages: polypropylene flasks, kraft paper bags and polyethylene nylon bags. After, they were conditioned in cold chamber at 16 ± 3°C and moisture content of 60 ± 3%, for a period up to 15 months with the following analyses in each three months.

### Germination test

Germination test was composed of four replications of 50 seeds without endocarps (parchments), totaling 200 seeds per treatment. It was assayed in germitest paper moistened with 2.5 times the weight of the paper at 30°C in germinator type B.O.D, with

evaluations in each 30 days until the end of the experiment. The final count of germination test was realized in the 30<sup>th</sup> day after the initiation of test, according to the recommendation of Rules for Seed Analysis (Brasil, 2009).

### Determination of moisture content

The method used involved oven drying at 105 ± 3°C for 24 h (Brasil, 2009). The samples were weighed with analytical scale of 0.001g of precision, with four replications of 50 g of seeds each.

### Evaluation of the length of primary root

Seven days after the initiation of germination test, the seeds were directed with the embryo down. On the 30<sup>th</sup> day, the measurements were taken using a graduated ruler, with distance between the final part of primary root to the collar region. The average length (cm) of roots was obtained by the division of summation of the measurements recorded by the number of roots.

### Count of filamentous fungus and yeasts

According to the methodology of MAPA (Brasil, 2003), the authors used a minimum of two decimal dilutions and one duplicate for each dilution. The incubation of plates was done at 25 ± 1°C, for five to seven days at B.O.D.

### Evaluation of the efficiency of products

The measurable response (dependent variable) was the count of colony forming units by amount of seeds (CFU g<sup>-1</sup> of seeds), expressed in terms of decimal reduction (g):  $Y = \log N_0/N$ , where: Y= number of decimal reductions achieved by treating, N<sub>0</sub>= initial number (CFU g<sup>-1</sup> of seeds) and N = number of survivors (CFU g<sup>-1</sup> of seeds). The results were expressed in colony forming units (CFU g<sup>-1</sup> of seeds).

### Identification of filamentous fungus

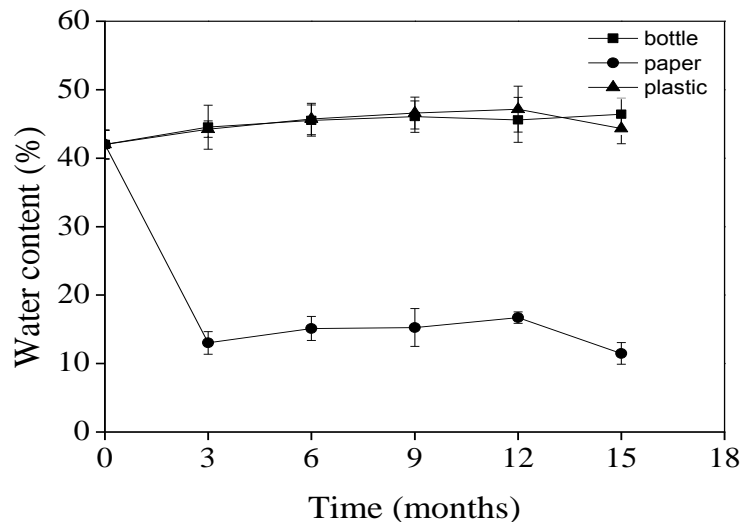
The morphology of vegetative and reproductive structures was observed on stereomicroscope and on light microscope. With the aid of dichotomous keys was realized the fungus identification at gender level and in some cases, after the determination of the gender, the collected material was compared with the descriptions of fungus published for the determination of species.

The experimental design used was completely randomized with four replications. The treatments were established in factorial scheme 16 × 3 × b5 (types of fungicides × types of packages × periods of evaluation). The average test used for the treatments was Dunnet unilateral at 5%, and interest is not in the differences between the treatments, but when there are treatments which are superior to the control and the maconzebe (reference treatment). To evaluate the effect of packages and the times, Tukey test at 5% was used. And for the microbiological analyses, the descriptive statistical was used.

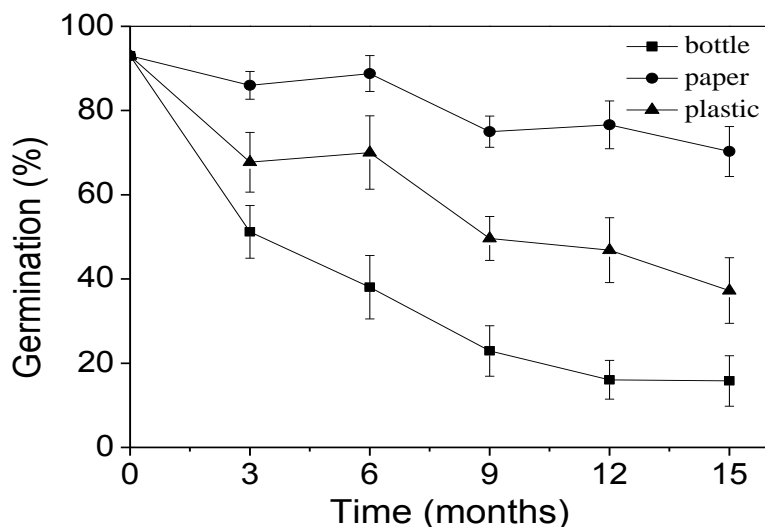
## RESULTS AND DISCUSSION

During the storage significant reduction was observed in the moisture content of the seeds conditioned in kraft paper, with moisture content of 14.3 ± 1.9%, while seeds





**Figure 1.** Moisture content of coffee seeds acondioned in different packages and stored in cold chamber for 15 months.



**Figure 2.** Germination of coffee seeds conditioned in different packages during 15 months ( $\pm$ standard error,  $n=16$ ).

conditioned in polypropylene flasks or in polyethylene nylon bags presented low reduction in the water content during the storage ( $45.6 \pm 4.1\%$  and  $45.6 \pm 6.1\%$ , respectively) (Figure 1). The variation in the water content of seeds was more intense in the package of paper making this kind of package to offers little resistance to the water vapor exchanges between the seeds and the environment. Similar results were found by Silva et al. (2010) and Alves and Lin (2003) with cotton seed.

Independently of the treatment, the paper package presented higher efficiency in the viability conservation of seeds, with germination above 70% at 15 months of storage to fungus control, possibly by the lower moisture content which does not favor the proliferation of

microorganisms capable to impair the germination. The high water content of stored seeds in others packages (flasks and plastic), may have negatively affected the germination of seeds to keep the metabolism of seeds more active, providing better conditions for the degradation of reserves and the microorganisms proliferation.

The type of package also affected the seeds from sixteen different treatments in relation to the viability conservation ( $p < 0.05$ ). Seeds conditioned in kraft paper presented percentage of germination significantly higher than those conditioned in flasks or in polyethylene nylon bags (Figure 2). The higher germination in seeds stored in this package may be related to the gas exchange

**Table 1.** Germination percentage (%) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products and medicinal plants aconditioned in polypropylene flasks, kraft paper bags and polyethylene+nylon bags.

Treatment	Polypropylene flasks					Kraft paper					Polyethylene + nylon bags				
	Months of storage					Months of storage					Months of storage				
	3	6	9	12	15	3	6	9	12	15	3	6	9	12	15
Control	85	81	46	--	--	84	84	57	51	31	81	80	54	51	--
Rosemary	92	97 <sup>C</sup>	89 <sup>CM</sup>	91 <sup>CM</sup>	86 <sup>CM</sup>	86	92	90 <sup>C</sup>	89 <sup>C</sup>	91 <sup>CM</sup>	94 <sup>C</sup>	95 <sup>C</sup>	93 <sup>C</sup>	91 <sup>C</sup>	87 <sup>C</sup>
Hoary Basil ( <i>Ocimum americanum</i> L.)	18	--	--	--	--	88	91	77 <sup>C</sup>	76 <sup>C</sup>	80 <sup>C</sup>	83	81	59	83 <sup>C</sup>	23 <sup>C</sup>
Garlic	94	90	87 <sup>CM</sup>	78 <sup>CM</sup>	79 <sup>CM</sup>	96 <sup>C</sup>	91	70 <sup>C</sup>	87 <sup>C</sup>	81 <sup>CM</sup>	90	88	78 <sup>C</sup>	93 <sup>C</sup>	86 <sup>C</sup>
Benzoate	--	--	--	--	--	87	85	59	75 <sup>C</sup>	64 <sup>C</sup>	35	--	--	--	--
Cinnamon	75	--	--	--	--	67	84	68	73 <sup>C</sup>	72 <sup>C</sup>	81	93 <sup>C</sup>	90 <sup>C</sup>	93 <sup>C</sup>	83 <sup>C</sup>
Horsetail	30	--	--	--	--	84	92	83 <sup>C</sup>	74 <sup>C</sup>	63 <sup>C</sup>	75	62	--	--	--
Clove	48	--	--	--	--	85	91	69 <sup>C</sup>	73 <sup>C</sup>	69 <sup>C</sup>	37	43	16	--	--
Fennel	--	--	--	--	--	80	88	69 <sup>C</sup>	68 <sup>C</sup>	70 <sup>C</sup>	73	43	--	--	--
Ginger	--	--	--	--	--	77	89	76 <sup>C</sup>	78 <sup>C</sup>	72 <sup>C</sup>	80	81	--	--	--
Basil ( <i>Ocimum basillium</i> )	19	6	--	--	--	84	81	68	86 <sup>C</sup>	77 <sup>C</sup>	13	24	22	--	--
Sorbate	--	--	--	--	--	93	91	78 <sup>C</sup>	54	--	--	--	--	--	--
Trichodel <sup>®</sup>	93	65	--	--	--	94	95 <sup>C</sup>	94 <sup>C</sup>	83 <sup>C</sup>	85 <sup>CM</sup>	88	94 <sup>C</sup>	76 <sup>C</sup>	90 <sup>C</sup>	89 <sup>C</sup>
Trichodermil <sup>®</sup>	89	79	--	--	--	94	86	73 <sup>C</sup>	86 <sup>C</sup>	76 <sup>C</sup>	94 <sup>C</sup>	86	80 <sup>C</sup>	83 <sup>C</sup>	93 <sup>CM</sup>
Trichoplus <sup>®</sup>	88	94 <sup>C</sup>	93 <sup>CM</sup>	88 <sup>CM</sup>	88 <sup>CM</sup>	90	96 <sup>C</sup>	87 <sup>C</sup>	91 <sup>C</sup>	92 <sup>CM</sup>	92 <sup>C</sup>	94 <sup>C</sup>	89 <sup>C</sup>	85 <sup>C</sup>	56 <sup>C</sup>
Mancozebe	91	92 <sup>C</sup>	52	--	--	--	88	86 <sup>C</sup>	84 <sup>C</sup>	72 <sup>C</sup>	92 <sup>C</sup>	95 <sup>C</sup>	88 <sup>C</sup>	84 <sup>C</sup>	81 <sup>C</sup>

\*C = significant (P < 0.05) in relation to the control and M = significant (P < 0.05) in relation to the Mancozebe by the unilateral test Dunnet; -- no germination.

capacity allowing the moisture equilibrium of the intern and the extern environment, favoring the reduction of seeds moisture (Figure 1). Like mentioned before, the reduction of moisture content could be disadvantage of the pathogenic microorganisms proliferation on seeds, allowing their higher germination as well as contributing to the cellular metabolism reduction of these seeds, what directly affecting the content of reserves used in the initial germination.

In relation to the microbiological control, high initial contamination of filamentous fungus and yeasts in coffee seeds ( $7.8 \times 10^5$  CFU g<sup>-1</sup> seeds)

was observed. The filamentous fungus predominant in coffee seeds were: *Penicillium* sp., *Aspergillus* sp., *Trichoderma* sp. and *Fusarium* sp. Seeds conditioned in flasks and treated with garlic, rosemary and Trichoplus<sup>®</sup> provided germination above 70% during all the period of evaluation with significant reduction in the total microbial, and the highest germinated at 12 and 15 months of stored (Tables 1 and 2). At nine months of storage, they were significantly higher than those treated with Mancozeb and the control, remaining like this until the end of the experiment (Table 1).

In paper kraft package, the higher reduction of fungal population was observed in seeds treated with garlic and rosemary. In the other treatments, including the control, there was increase of fungal population and reduction in the germinative power of seeds (Table 3). In plastic bags packages, better results in the reduction of microbiological population was also observed in treatments with garlic and rosemary. In other treatments, including the control, there was increase in the fungal population during the storage (Table 4).

The use of vegetative extracts, mainly garlic and rosemary, were efficient in the control of

**Table 2.** Decimal reductions (Y) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products, and medicinal plants aconditioned in polypropylene flasks.

Treatment	Months of storage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Control	-0.81	-1.56	-1.56	-2.04	--
Rosemary	0.46	0.48	0.78	1.02	2.59
Hoary Basil ( <i>Ocimum americanum</i> L.)	-0.89	-2.15	--	--	--
Garlic	0.39	0.57	1.41	1.46	1.44
Benzoate	1.85	--*	--	--	--
Cinnamon	1.32	-1.31	--	--	--
Horsetail	-1.11	0.11	--	--	--
Clove	2.89	3.85	--	--	--
Fennel	-2.06	-2.63	--	--	--
Ginger	-1.39	-2.43	--	--	--
Basil ( <i>Ocimum basillium</i> )	-2.11	-1.89	-1.91	--	--
Sorbate	2.89	--	--	--	--
Trichodel®	3.90	-0.77	-3.22	--	--
Trichodermil®	2.89	4.94	4.94	--	--
Trichoplus®	0.46	0.36	0.85	0.24	0.75
Mancozebe	1.34	2.41	2.61	2.11	--

\*--Germination equal to zero in period of anterior evaluation, however without microbiological evaluation.

**Table 3.** Decimal reductions (Y) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products and medicinal plants dehydrated, powdered and aconditioned in kraft paper bags.

Treatment	Months of storage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Control	0.48	0.18	0.06	-0.01	0.10
Rosemary	0.34	0.44	0.99	0.59	1.30
Hoary Basil ( <i>Ocimum americanum</i> L.)	-0.97	0.59	-0.45	0.20	-0.01
Garlic	0.55	0.37	1.57	1.53	1.72
Benzoate	1.31	2.16	2.35	2.41	1.51
Cinnamon	2.99	1.69	-0.36	1.29	2.49
Horsetail	0.41	-0.78	0.05	-0.19	-0.39
Clove	2.89	4.94	2.92	4.59	4.94
Fennel	-1.15	-0.52	-1.22	-1.69	-1.87
Ginger	-0.22	-0.36	-0.22	-0.52	0.55
Basil ( <i>Ocimum basillium</i> )	-1.15	-0.54	-0.22	-0.49	0.05
Sorbate	2.89	3.90	3.59	3.43	--
Trichodel®	3.90	0.19	-2.71	-0.11	--
Trichodermil®	2.89	4.94	4.94	4.94	4.94
Trichoplus®	0.29	0.14	0.55	0.10	0.14
Mancozebe	1.17	0.95	2.14	2.02	1.49

-- Germination equal to zero, so there was no possible measure for the decimal reduction.

**Table 4.** Decimal reductions (Y) of coffee seeds (*Coffea arabica*) treated with biological products, chemical products, and medicinal plants dehydrated and powdered aconditioned in polyethylene+ nylon bags.

Treatment	Months of storage				
	3	6	9	12	15
	Y	Y	Y	Y	Y
Control	-0.56	0.48	0.81	1.31	-0.90
Rosemary	0.55	0.49	1.19	0.85	2.69
Hoary Basil ( <i>Ocimum americanum</i> L.)	1.81	-1.22	-1.01	0.29	-0.41
Garlic	0.51	0.20	1.41	1.49	1.18
Benzoate	1.99	0.53	0.69	1.49	--
Cinnamon	0.11	0.29	0.61	0.46	1.14
Horsetail	-1.15	1.11	-1.90	-2.28	--
Clove	2.89	2.51	3.16	2.36	--
Fennel	-1.41	-2.01	-1.47	--	--
Ginger	-1.25	-1.57	-1.96	-3.83	--
Basil( <i>Ocimum basillium</i> )	-1.11	-1.28	-0.47	0.99	--
Sorbate	2.89	3.90	4.94	--	--
Trichodel <sup>®</sup>	3.90	-0.19	-2.65	-1.96	--
Trichodermil <sup>®</sup>	2.89	4.94	4.94	4.94	4.94
Trichoplus <sup>®</sup>	0.75	0.59	0.89	0.55	0.85
Mancozebe	2.26	1.95	2.85	2.09	1.12

\*-- Germination equal to zero, so there was no possible measure for the decimal reduction.

microorganisms by having substances like terpenoids, essentials oils, alkaloids (Barrera-Necha et al., 2008), which can act in the microorganisms control. Silva et al., (2012) verified that the aqueous extract of garlic promoted a relative inhibition of the development *in vitro* phytopathogenic mycelium. In the present study, garlic presented excellent fungus control, which can be related to their chemical composition.

Several studies show that allicin, S-allyl cysteine, Allyl cysteine Mercapto, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide are volatile compounds presented in garlic and which presents antioxidant activity (Cecilia and Olubunmi, 2014; Dias et al., 2011; Queiroz et al., 2006). These antioxidant compounds may have influenced the maintenance of seeds quality, not just controlling the proliferation of microorganisms, but also contributing to the preservation of germination of seeds by acting on reactive oxygen species which generate oxidation chain reactions and affecting the reserve compounds, membranes, genetic material between others (Cecilia and Olubunmi, 2014; Perelló et al., 2013; Baraka et al., 2011; Santos et al., 2010)

In relation to rosemary product, the  $\alpha$ -pineno, 1,8-cineol and the camphor, major constituents of the essential oil (Ribeiro et al., 2012), are the compounds that may have assisted in maintaining the viability of the seeds. According to the same authors, these compounds present inhibitory effects on fungus and bacteria growth, which can explain the efficiency of these treatments in

the maintenance of viability of coffee seeds in the present study. Brand et al. (2010) verified that higher reduction occurs in growth of fungus and increase in the viability of coffee seeds, using autoclaved aqueous extract of rosemary in doses of 2.5%. Similar results were observed by Souza et al. (2010) using rosemary extract in different concentrations in seeds of *Inga edulis*. This fungicide effect of extract, has effect on many microorganisms (bacteria and fungus) which can affect several forms of the physiological quality and, in some cases, completely inhibit the germination of seeds (Lopes et al., 2011; Neto et al., 2012.).

Leite et al. (2012) showed that the use of vegetal extract did not affect the seeds metabolism of *Mimosa caesalpiniaefolia* Benth and even favored the control of pathogens, increasing the viability of seeds. The same behavior related to the speed of germination was also reported by Xavier et al. (2012), in seeds of cowpea-beans (*Vigna unguiculata* L.). Between the biological products, Trichodermil<sup>®</sup> and Trichoplus<sup>®</sup> were more effective in the reduction of fungus population in all the packages. This efficiency can be attributed to several mechanisms of action used by fungus, like the production of metabolites and enzymes with antifungal properties, the hyperparasitic and the competition for nutrients (Harman et al., 2004), and avirulent symbionts associated with plants (Carvalho et al., 2011). It is also suggested that they have effect like growth vegetal stimulators and also the production of auxin analogs

(Harman et al., 2004; Vinale et al., 2008).

## Conclusion

The paper packaging is best suited for the preservation of coffee seeds, independent of the microorganism control treatment used. Garlic, rosemary and organic products are effective in controlling microorganisms and maintenance of coffee seed viability stored for 15 months with higher germination required for minimum of 70%.

## Conflict of interest

The author has not declared any conflict of interest.

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*Full Length Research Paper*

## Grain sorghum leaf reflectance and nitrogen status

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**Nitrogen deficiency is a common but readily managed constraint to grain yield. A quick and non-destructive detection of crop N status using remote sensing could be a means to increased N use efficiency. Research was conducted in a greenhouse in 2006 at the University of Nebraska-Lincoln to establish the relationship of spectral reflectance with N status in leaves of grain sorghum, to develop indices for interpretation of the results and to predict chlorophyll content. Nitrogen stress decreased chlorophyll meter reading and leaf N content, but increased leaf and canopy reflectance. The SPAD values were significantly increased by both water and N stress. Reciprocal reflectance in the green range (549 to 560 nm), and red edge range (710 to 718 nm) wavelength of the spectrum were good indicators of N stress. The best fit regression between leaf chlorophyll content and the indices in the green and red edge wavebands were linear with an R<sup>2</sup> of 0.76 to 0.79. A model calibrated using these wavelengths minus reciprocal reflectance of NIR (750 nm), predicted leaf chlorophyll content with root mean square error (RMSE) ranging between 52 and 56 mg m<sup>-2</sup>, and reduced the intercept of the model from 312 to 35 mg m<sup>-2</sup> in the green range and 486 to 21 mg m<sup>-2</sup> in the red edge. Future studies will be conducted to evaluate the effectiveness of the indices at the canopy level of grain sorghum.**

**Key words:** Chlorophyll, grain sorghum, nitrogen, red edge, reflectance, SPAD.

### INTRODUCTION

Sorghum (*Sorghum bicolor* (L) Moench) is the fifth most important cereal after rice, wheat, maize, and barley. Sorghum is a major food grain for millions in the semi-arid tropics of Africa, Asia, and Latin America and is an important commercial and export crop in the USA, Argentina and Australia (US Grain Council, 2015). Improved varieties respond to N and water stresses as any of the other cereals (Maman et al., 2003; Zhao et al., 2005). It is more tolerant of drought and nutrient stresses

than some other cereals and often well-adapted to semi-arid conditions. Nitrogen availability is often inadequate for optimum grain sorghum yield but lack of available water reduces N uptake and decreases yield response to N (Ferguson, 2000).

Determining N status by remote sensing is one tool for improving N management and yield predictions in many crops. When radiation corresponding to the wavelengths of pigment absorption bands is incident upon green

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vegetation, the reflectance is reduced to a varying extent, depending on the tissue pigment content. Absorption by water and pigments determine to a large extent the reflectance spectrum of a leaf. Chlorophyll and accessory pigments absorb strongly between 400 and 700 nm.

Several studies have reported the use of remote sensing to quantify N stress in many plant species and predict crop yields (Graef and Claupein, 2003; Schlemmer et al., 2005; Gitelson et al., 2005; Zhao et al., 2007). Simple ratio (SR) and normalized difference vegetation index (NDVI) have been widely used to assess ground coverage of plant vegetation, leaf area index (LAI), biomass production and crop yields (Aparicio et al., 2000, Moges et al., 2007). However, other studies have found that NDVI saturates with high biomass and is insensitive to variations in chlorophyll concentration on reaching saturation at low LAI (Aparicio et al., 2000; Daughtry et al., 2000). The use of red-edge reflectance rather than red has improved the early detection of plant stress and crop yield estimation (Gitelson et al., 2005; Eitel et al., 2009; 2011).

Only a few of these indices have been tested on grain sorghum (Mandal et al., 2007; Moges et al., 2007; Zhao et al., 2005). In addition few of these studies considered the combined effect of water and N availability and its effects on leaf reflectance of grain sorghum. Richardson et al. (2002) cautioned that differences in leaf structure may necessitate species-specific calibration equations.

## Hypothesis and objectives

Leaf and canopy reflectance of grain sorghum can be used to evaluate N stress. The relationships between leaf and canopy spectral reflectance and water and N stresses of grain sorghum were evaluated. Specific objectives of the study were:

- i. Determine reflectance patterns of sorghum leaf exposed to N and water stress.
- ii. Identify spectral bands in which leaf reflectance were most affected by N content.
- iii. Calibrate and validate spectral indices for the detection of N stress in grain sorghum at leaf level and compare these with published indices.

## MATERIALS AND METHODS

The effect of water and N stresses on spectral reflectance of grain sorghum was addressed in a greenhouse study conducted from February to April, 2006. Forty five (45) pots with capacity of 9.45 L were filled with equal volumes of Crete silt loam mixed with sand. Inorganic N as urea was applied at the equivalent of 0, 34, 68, 100 and 135 kg N ha<sup>-1</sup> with 50% applied pre-planting and 25% each at 28 and 42 days after emergence to minimize leaching of nitrate-N from the pots. Phosphorus was applied a 45 kg P ha<sup>-1</sup> in the form

of triple super-phosphate and potassium at 20 kg K ha<sup>-1</sup> in the form of muriate of potash before planting. A medium maturity sorghum hybrid, Dekalb 42-20, was planted and thinned to leave three plants per pot after emergence. A completely randomized design (CRD) with four replications was used. Twelve-hour (7am to 7pm), 400 watt incandescent light was used and temperature was kept at 29°C for day and 18°C for night temperature.

Three levels of soil water matric potential were imposed beginning 32 days after emergence (DAE): adequate/low soil water stress (LOW; <20 kPa); medium water stress (MEDIUM; 40<kPa<80), and high water stress (HIGH; >100 kPa). Soil matric potential was recorded with Watermark sensors (Irrometer Co., Riverside, CA, USA) installed in the middle of each pot. Each Watermark sensor was connected to a data logger and soil water matric potential was logged hourly. At 75 DAE, Minolta SPAD-502 (SPAD) chlorophyll meter, SPAD (Minolta Co., Osaka, Japan) readings were taken from the middle section along the length and midway between the margin and the midrib of the most recently fully expanded leaf. Two measurements were taken from each of three leaves for each pot; afterwards these leaves were removed, kept in a polyethylene bag under ice, and sent to the laboratory for reflectance measurement and chlorophyll extraction (Daughtry and Biehl, 1985). Plants in each pot were harvested, weighed immediately, and then dried at 70°C for 72 h to determine dry weight.

## Reflectance measurement, relative water content and chlorophyll extraction

Before chlorophyll extraction, spectral reflectance of the three previously used leaves for SPAD measurement was measured with an ASD Fieldspec FR spectroradiometer (Analytical Spectral Device, Boulder, CO) connected to a Li-COR integrating sphere (LI-COR Inc., Lincoln, NE). A BaSO<sub>4</sub> reference was used to calibrate all reflectance measurements. Six scans were taken per leaf. Each spectral scan measured the reflectance from 350 to 2500 nm at 1-nm increments. The spectral data was converted to reflectance and the data above 2200 nm was discarded due to high noise to signal ratio.

Relative water content (RWC) and total chlorophyll content of the incised leaves were determined. Ten 1 cm disks were taken from each leaf that was used for spectral reflectance measurement. Five disks were selected randomly and weighed immediately providing a measure of fresh weight ( $L_f$ ). The leaf disks were soaked in deionized water for 24 h and then weighed again to obtain the turgid weight ( $L_t$ ). Finally, the leaf disks were dried at 85°C and weighed to obtain a dry mass ( $L_d$ ). The RWC was calculated (Salisbury and Ross, 1992) as:

$$RWC = (L_f - L_d) / (L_t - L_d).$$

The remaining set of five leaf disks were used to determine chlorophyll content using the dimethyl sulphoxide (DMSO) chlorophyll extraction technique (Barnes et al., 1992). Ten milliliters of DMSO and leaf disks were placed in a 65°C water bath for 30 min. The DMSO extract was read on a DU 800 spectrophotometer to acquire absorption ( $A_\lambda$ ) measurements at 500 to 750 nm wavelength, which was used to calculate chlorophyll concentration ( $Chl_{conc}$ ). Equations for Chl *a* and Chl *b* as provided by Wellburn (1994):

$$\begin{aligned} Chl\ a &= 12.19A_{665} - 3.45A_{649}; (\mu g\ ml^{-1}) \\ Chl\ b &= 21.99A_{649} - 5.32A_{665}; (\mu g\ ml^{-1}) \\ Total\ Chl_{conc} &= Chl\ a + Chl\ b; (\mu g\ ml^{-1}) \end{aligned}$$

**Table 1.** ANOVA summary for a greenhouse study with fertilizer N rates under different levels of soil water stress at 75DAP.

SOV	df	†DMY	Chl	SPAD	RWC	R <sub>549-560</sub>	R <sub>710-718</sub>	R <sub>1450-1460</sub>	R <sub>1760-1770</sub>
Units		---g---	mg m <sup>-2</sup>		-%-	-----%-----			
-----Mean Square(MS)-----									
N rate (N)	4	623**	105063**	363**	0.205**	68.0 **	76.9**	12.8**	8.10**
Water level (W)	2	132**	809.5ns	7.2**	0.084**	20.1**	19.5**	14.8**	10.9**
N*W	8	21.3**	3411**	11.1**	0.014ns	2.78ns	4.61ns	4.95**	3.74**
Residual	28	0.83	377	0.92	0.002	2.45	2.57	1.74	0.81

†Dry matter yield (DMY), total leaf chlorophyll (Chl), SPAD values (SPAD), relative leaf water content (RWC). ‡Reflectance, R at average of 549 to 560, 710 to 718, 1450 to 1460 and 1760 to 1770 nm wavelength. \*Significant at 5%, \*\* Significant at 1% or less, ns Not Significant.

Chlorophyll content was derived as a function of chlorophyll concentration, the volume of DMSO (DMSO<sub>vol</sub>) used in the extraction, and the leaf disk area (LDA) sampled:

$$\text{Chlorophyll (Chl) content} = (\text{total Chl}_{\text{conc}} * \text{DMSO}_{\text{vol}}) / \text{LDA}; (\text{mg m}^{-2})$$

#### Indices calibration and validation

Using the concept proposed by Gitelson et al. (2003), four indices were calibrated as follows:

- i.  $R_{[\text{green}]}^{-1}$
- ii.  $R_{[\text{green}]}^{-1} - R_{[\text{NIR}]}^{-1}$ ,
- iii.  $R_{[\text{RE}]}^{-1}$ ,
- iv.  $R_{[\text{RE}]}^{-1} - R_{[\text{NIR}]}^{-1}$ ,

Where  $R_{[\lambda]}^{-1}$  is the reciprocal reflectance of green, red edge (RE) and near infrared (NIR) and compared with three published frequently used indices listed as follows:

$$\begin{aligned} \text{Simple ratio index (SR)} &= R_{\text{NIR}}/R_{\text{RED}} \text{ (Rouse et al., 1974),} \\ \text{NDVI} &= (R_{\text{NIR}} - R_{\text{RED}})/(R_{\text{NIR}} + R_{\text{RED}}) \text{ (Rouse et al., 1974),} \\ \text{GNDVI} &= (R_{\text{NIR}} - R_{\text{GREEN}})/(R_{\text{NIR}} + R_{\text{GREEN}}) \text{ (Gitelson et al., 1996).} \end{aligned}$$

#### Data analysis

All data were analyzed by analysis of variance mixed linear model procedure (Proc Mixed, SAS Institute, 2007, Cary, NC, USA). Where the F test was significant at  $P \leq 0.05$ , the least significant difference (LSD) was calculated and used to separate treatment means. Regression analysis was performed to establish relationships of reflectance with SPAD values, chlorophyll content and biomass yield. The data collected from the experimental setup was divided into two. The data from one set was used to calibrate the reflectance indices and the second data set was used for the indices validation.

## RESULTS AND DISCUSSION

### Relationship of SPAD values with chlorophyll content and dry matter yield

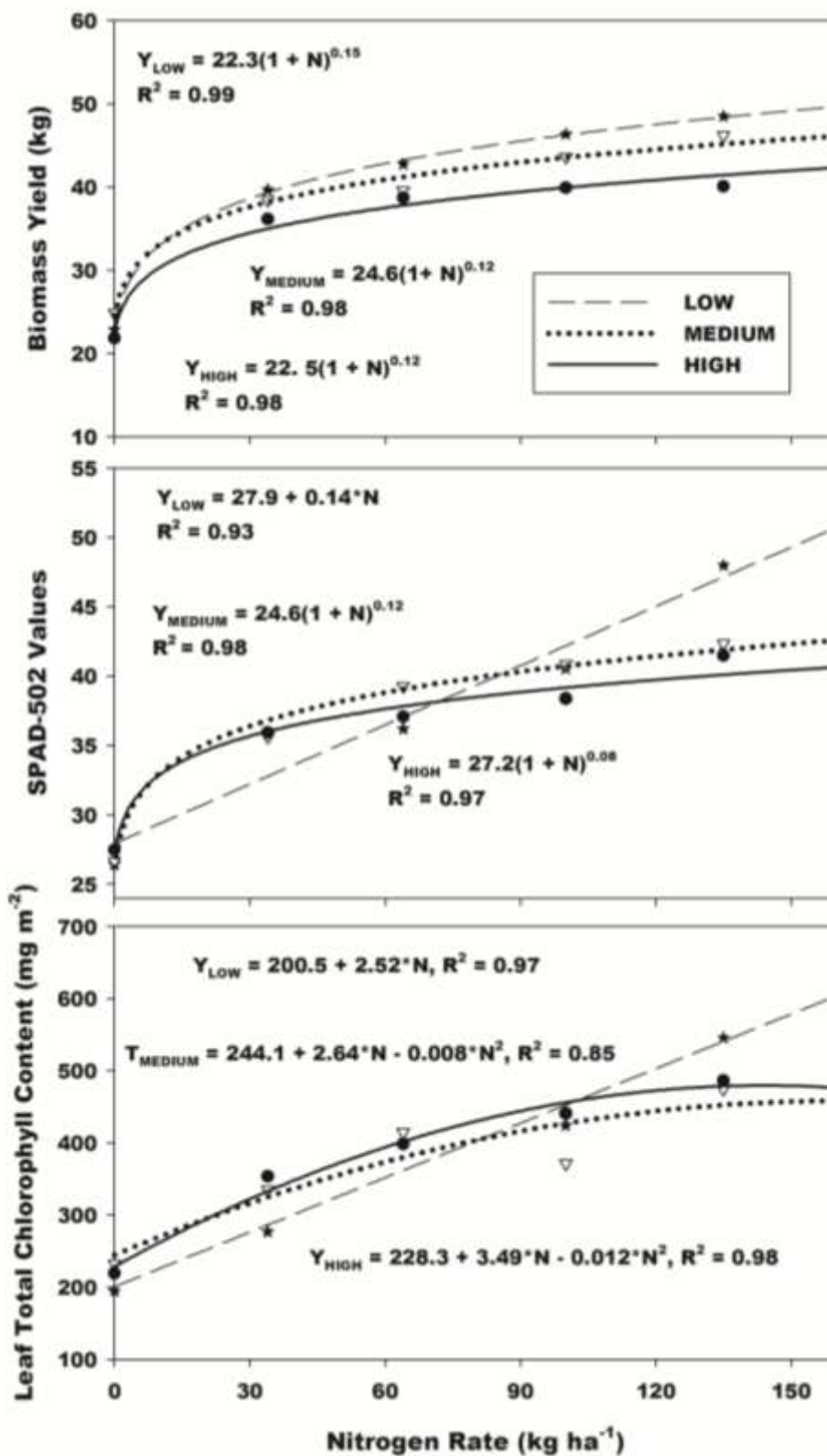
The interaction between N rate and water-stress levels

significantly influenced dry matter yield, chlorophyll content, SPAD values, and the reflectance mean of 1450 to 1470 nm and mean of 1760 to 1770 nm (Table 1). Water-stress level resulted in significant differences in all parameters measured, except leaf total chlorophyll content.

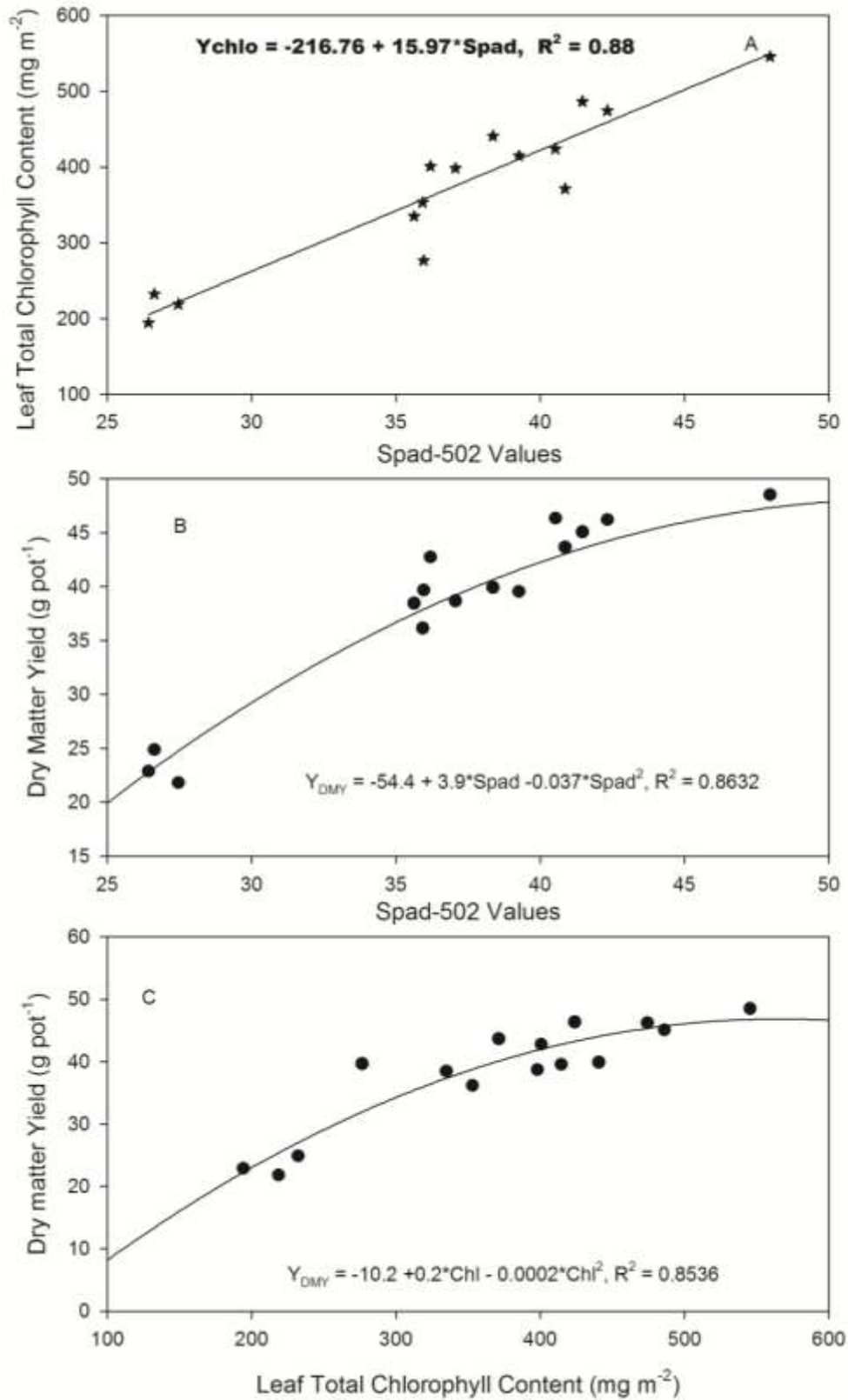
At each soil water level, the best fit function of the relationship between N rate and biomass yield was a power function with  $R^2$  values of 0.99 with Low and 0.98 with Medium and High water-stress levels (Figure 1A). Water stressed plants responded less to increased N rate and the response to N leveled off after 60 kg N ha<sup>-1</sup>. Medium and Low water stressed plants reached a plateau between 90 and 120 kg N ha<sup>-1</sup>. At any N rate, stressed plants had lower yield per kg N applied compared to Medium and Low treatment. Several studies have reported significant water and N interactions on growth and development and biophysical characteristics of crops (Schepers et al., 1996; Martinez and Guiamet, 2004; Zhao et al., 2005).

With an adequate water supply, SPAD values and chlorophyll content had a positive linear relationship with N rate, while the best fit function was power for Medium and High stressed conditions with  $R^2$  values of 0.92 with Medium and 0.98 with High (Figure 1B). With Low water stressed, the relationship between leaf chlorophyll and N rate was linear with  $R^2$  value of 0.97. While with Medium and High, quadratic functions with  $R^2$  values of 0.84 and 0.98 with Medium and High described the relationship between leaf chlorophyll and N rate (Figure 1C).

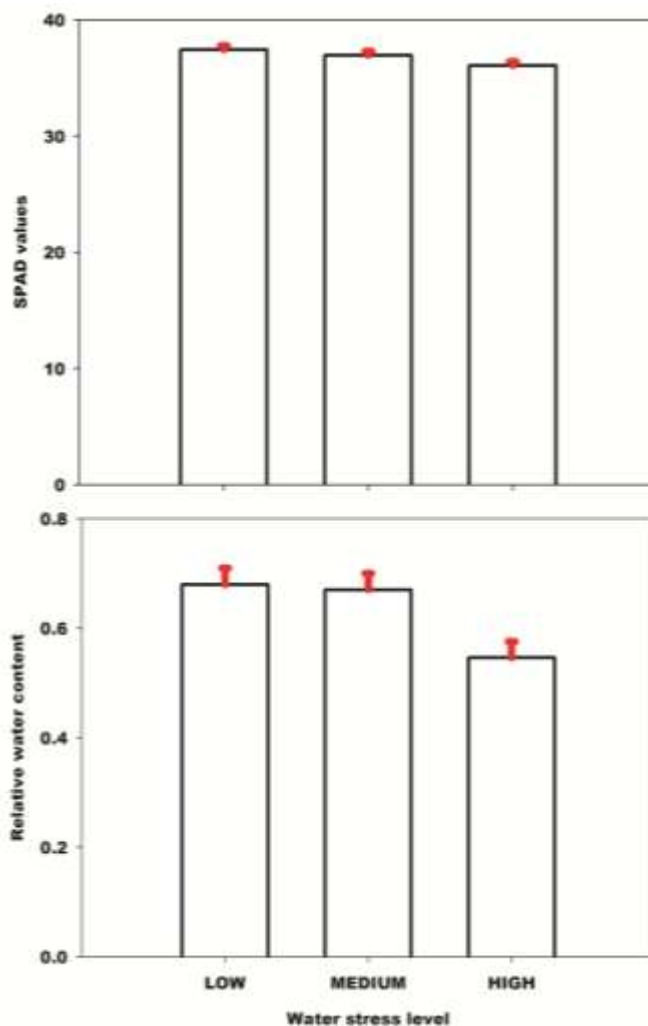
There was a quadratic relationship between SPAD and chlorophyll content with chlorophyll being increased with increasing SPAD and a coefficient of determination of 0.88 (Figure 2A). Significant relationships between SPAD values and chlorophyll content have been reported by Markwell et al. (1995), Schepers et al. (1996) and Martinez and Guiamet (2004). Since much of leaf N is incorporated in chlorophyll, chlorophyll in leaves has been used to assess N status of crops. Chlorophyll meters have been used to detect N stress in corn (Zea



**Figure 1.** Effect of N rates and water stress levels on grain sorghum biomass yield (A) SPAD values (B) and total chlorophyll concentration (C) at 75 days after emergence in a greenhouse. LOW, MEDIUM and HIGH water stress.



**Figure 2.** Best fit relationship of SPAD values with grain sorghum leaf total chlorophyll content (Chl) (A) SPAD with dry matter yield (B) and of Chl with dry matter yield (C) across five N rates and three water levels at 75 days after planting in a greenhouse

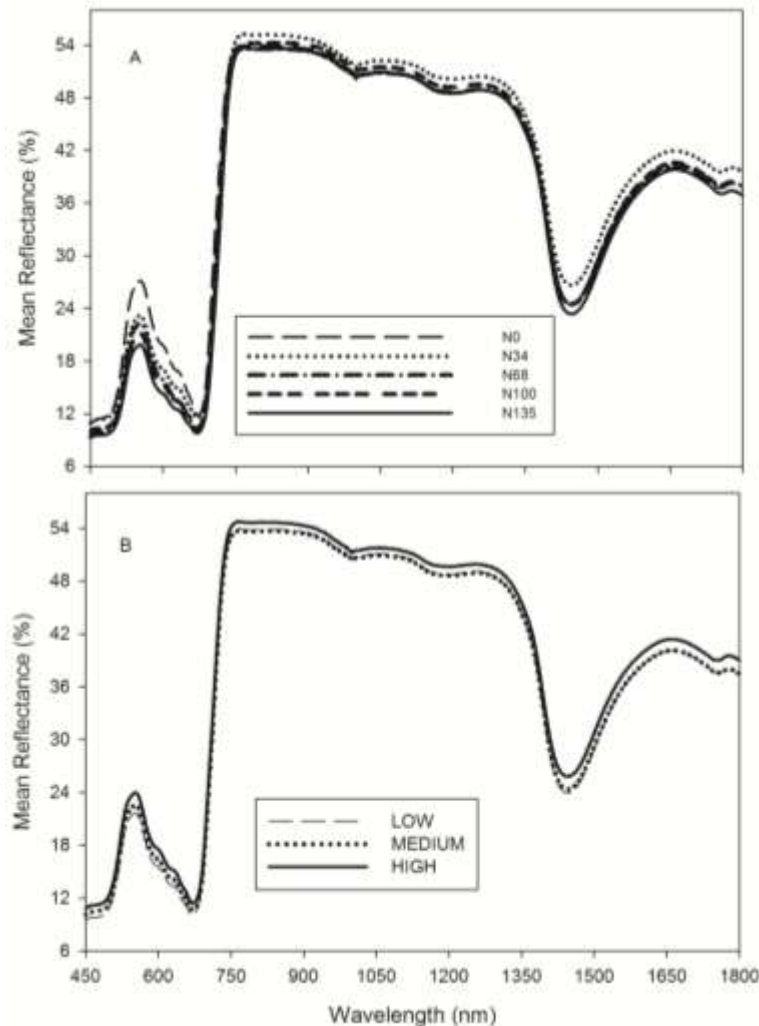


**Figure 3.** Effect of soil water on leaf SPAD values and relative leaf water content of grain sorghum in a greenhouse study. Y-bars = LSD (0.05).

*mays* L.) leaves (Schepers et al., 1992; Wood et al., 1992; Blackmer et al., 1994). The increase in dry matter yield relative to SPAD and chlorophyll content was quadratic with  $R^2$  of 0.86 and 0.85, respectively (Figure 2B and C). Although SPAD measurements are rapid and easy, the measurement represents a very small portion of a leaf. Water stress, leaf age and time of the day influence SPAD readings (Schepers et al., 1996; Martinez and Guiamet, 2004; Schlemmer et al., 2005). The use of SPAD in predicting leaf N status in grain sorghum and other plants must be guided by water status of the crop since SPAD tends to under-estimate leaf N status under water stress (Schepers et al., 1996; Schlemmer et al., 2005).

In this study, water stress decreased SPAD values and reduced the relative water content of the leaf (Figure 3).

Relative water content (cell turgor) in plants growing under field conditions varies during the day. Martinez and Guiamet (2004) observed that SPAD values were increased when a maize leaf was dehydrated and reduced when the same leaf was re-hydrated in a laboratory. However, Schepers et al. (1996) and Schlemmer et al. (2005) reported that water stress in maize leaves reduced SPAD values. Water stress in plants reduces RWC and cell turgor and this increases transmittance of the near infrared energy through the leaf tissue. The intercellular air spaces in the leaf tissue are influenced by cell turgor which is directly influenced by plant water status. The SPAD output is a function of leaf transmittance in the red and NIR (650 and 940 nm) wavelengths and is affected by changes in the intercellular air spaces of the leaf.



**Figure 4.** Effect of water (A) and nitrogen (B) stress on leaf spectral reflectance of grain sorghum.

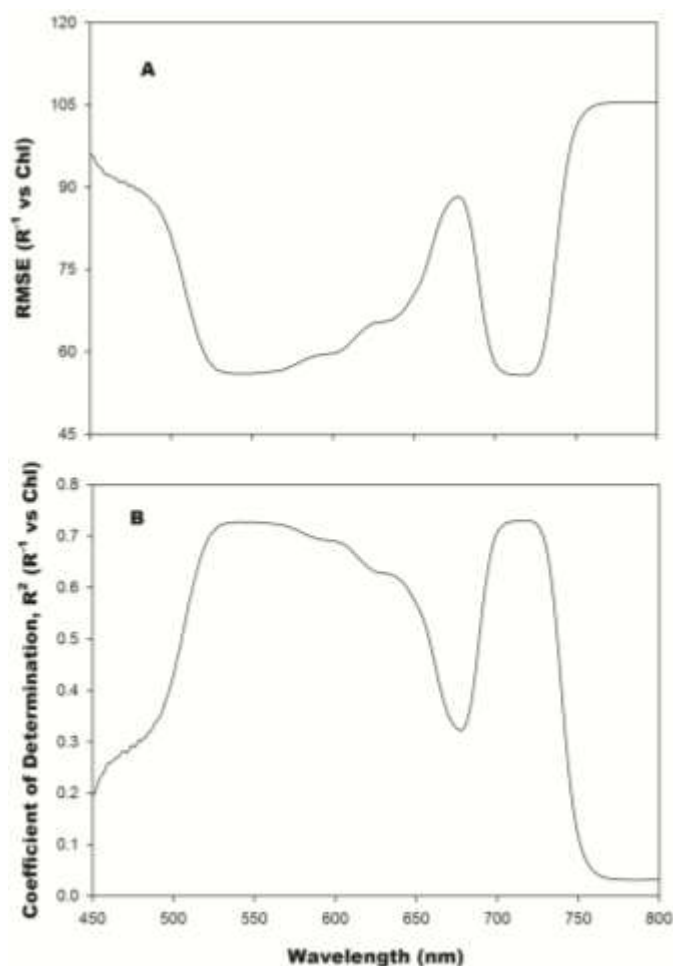
### Effect of water and N stresses on leaf spectral reflectance

Water  $\times$  N rate interaction resulted in differences in spectral reflectance in the mid-infrared (1450 to 1460 and 1760 to 1770 nm) range (Table 1). In general, inadequate water and N caused increased reflectance in the visible, near infrared (NIR) and mid infrared (MIR) regions of spectral profile for grain sorghum (Figure 4A and B).

In sections of green (549 to 560 nm), red edge (710 to 717 nm) and MIR (1450 to 1460 nm and 1760 to 1770 nm), water and N stress significantly affected the spectral reflectance of sorghum (Table 1). Many environmental and physiological factors can cause increased leaf reflectance, but N deficiency generally increases reflectance in the green and the red edge ranges (Carter and Knapp, 2001; Daughtry et al., 2000; Zhao et al.,

2003). Leaf reflectance in the green, red edge and MIR wavelength of the spectrum can be good indicators of N and water stress in plants (Graef and Claupein, 2003; Schlemmer et al., 2005; Gitelson et al., 2005; Zhao et al., 2007). Reflectance properties of leaves are controlled by the absorption and scattering processes which occur within the leaf. Light is reflected (scattered) at the interface of media with different reflective indexes such as cell wall-air interfaces in the intercellular spaces inside the leaf.

Chlorophyll content was generally high with all N rates (0 to 150 kg N ha<sup>-1</sup>), ranging from 200 to 500 mg m<sup>-2</sup> (Figure 1C). Variability in chlorophyll content with fertilizer N rate has been observed in other studies on maize and wheat which perhaps are more sensitive to N stress (Schepers et al., 1996; Schlemmer et al., 2005). Sorghum leaf reflectance in the green and red edge



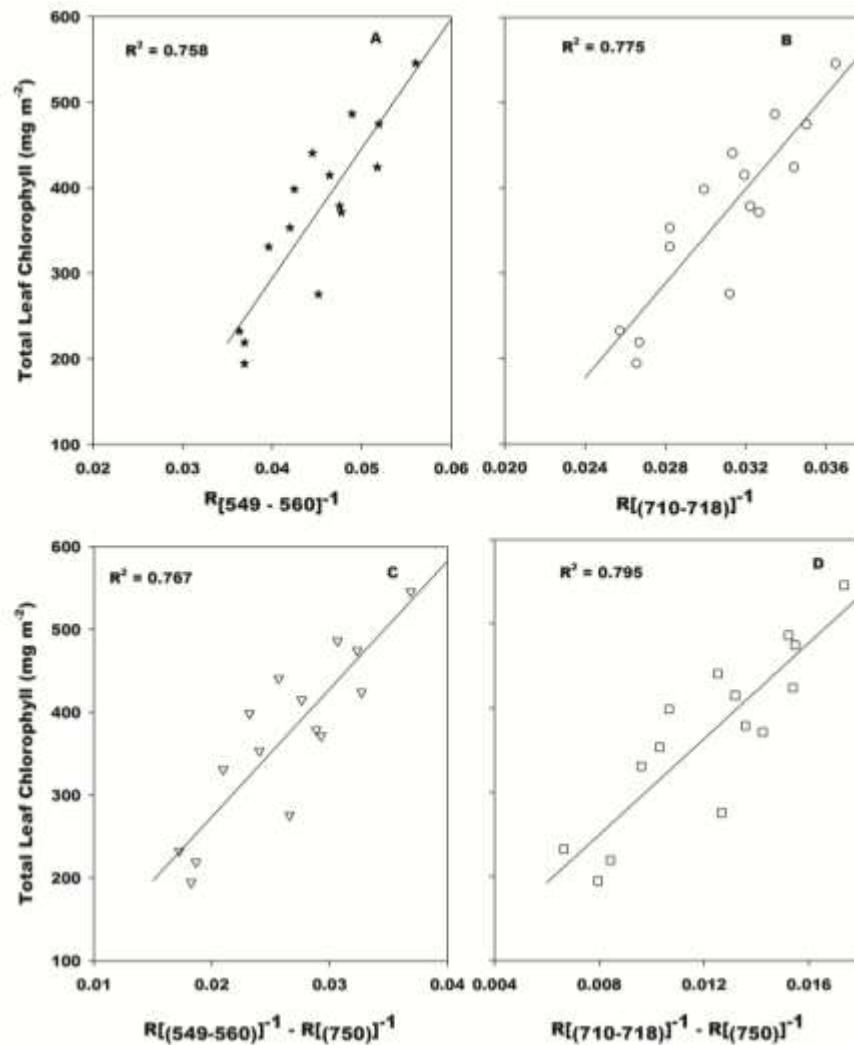
**Figure 5.** Coefficient of determination,  $R^2$  (A) and root mean square error, RMSE (B) of relationship between reflectance and leaf chlorophyll, and wavelength

wavelengths had the best correlation with chlorophyll content with an  $R^2$  value of 0.74, compared with lower  $R^2$  values of 0.21 in the blue, 0.36 in the red and 0.20 in the NIR wavelength. There is strong absorption of biochemical pigments for photosynthetic activities in the blue and red spectral region. According to Gitelson (personal communication), even in completely yellow leaves, absorption is higher than 85% in the blue spectral region. Due to high reflectance in the green spectral region, the region is sensitive to wide ranges of chlorophyll content, hence the strong coefficient of determination observed. Sims and Gamon (2002) reported that reflectance around the 700 nm spectral region was the most sensitive indicator of chlorophyll of many non-related leaves and that the ratio of NIR to red edge indices proposed by Gitelson and Merzlyak (1994) could be used as a measure of chlorophyll content for many plant species.

### Reflectance indices calibration and validation

Using the concept developed by Gitelson et al. (2003), a model was calibrated and validated with an independent data set for leaf chlorophyll content estimation in grain sorghum in a greenhouse study. According to Gitelson et al. (1996), reciprocal reflectance alone at certain wavelengths could be used to quantitatively estimate chlorophyll content. In order to be able to select a spectral range that could be used to calibrate a model for leaf chlorophyll content estimation, a linear correlation between chlorophyll content and spectral reflectance was established. The wavelength with the lowest root mean square error (RMSE) and highest  $R^2$  in the visible and RE regions and the wavelength with the highest RMSE and lowest  $R^2$  in the NIR regions were selected for the calibration (Figure 5). Reciprocal reflectance at 549 to 560 nm ( $R_{[549-560]}^{-1}$ ) with the peak at 550 nm ( $R_{[550]}^{-1}$ ) in





**Figure 6.** Relationship between reflectance index ( $R_x$ )<sup>-1</sup> and leaf total chlorophyll content in green (A) and RE (B) spectral range, and subtraction NIR ( $R[(750)]$ )<sup>-1</sup> from ( $R_x$ )<sup>-1</sup> green (C) and red edge (D).

the green spectral range and from 710 to 718 nm ( $R[(710-718)]$ )<sup>-1</sup> with a peak at 718 nm ( $R[(718)]$ )<sup>-1</sup> in the red edge range were selected for model calibration since this relationship had the highest  $R^2$  and lowest RMSE in the spectral profile and agreed with Gitelson et al. (2003). The best fit regressions between chlorophyll contents and the four reflectance indices were linear with an  $R^2$  of 0.76 to 0.79 (Figure 6).

According to Gitelson et al. (2003),  $R[(NIR)]$ <sup>-1</sup> values are comparable to chlorophyll content in leaves with very low chlorophyll content and thus represent scattering and non-pigment leaf absorption. Subtracting  $R[(NIR)]$ <sup>-1</sup> values from the green and RE index slightly improved  $R^2$  values and significantly reduced the intercept of the model from 312 to 35  $\text{mg m}^{-2}$  in the green range and from 486 to 21  $\text{mg m}^{-2}$  in the RE range (Table 2). Zhao et al. (2005)

suggested two narrow ranges centered on  $R_{(555)}$  nm and  $R_{(715)} (\pm 5)$  nm for detecting N deficiency in sorghum. They found the ratio of two indices  $R_{1075}/R_{735}$  and  $R_{405}/R_{715}$  had a better linear relationship than single waveband indices with  $R^2$  values ranging from 0.64 to 0.82.

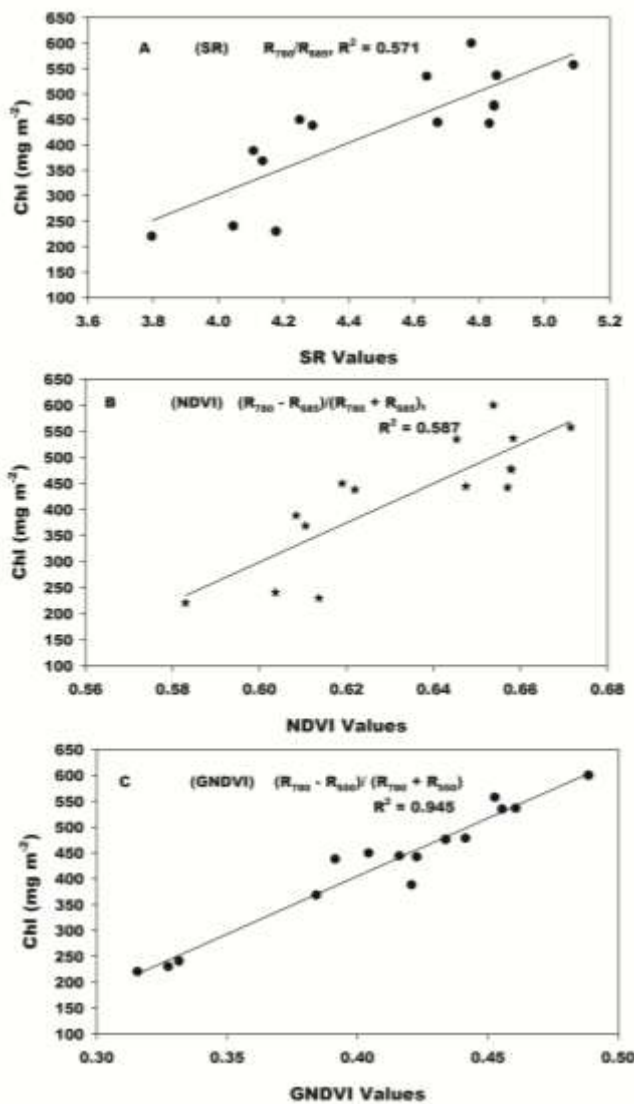
The normalized difference vegetation index (NDVI) and simple ratio (SR) index (Rouse et al., 1974; Aparicio et al., 2000) are two commonly used vegetative indices in remote estimation of chlorophyll in plants. These two indices and the green NDVI (Gitelson et al., 1996) were compared with reciprocal reflectance indices suggested in this study using the same data set. Both NDVI and SR performed poorly, while GNDVI did better compared to the suggested indices in estimating chlorophyll (Figure 7). Gitelson et al. (2003) reported that indices that use



**Table 2.** Calibrated models for estimating total leaf total chlorophyll (Chl) content in grain sorghum leaves at 75 days after planting in a greenhouse study.

$\dagger R_\lambda$	Model	$R^2$
$R_{[549-560]}^{-1}$	$Chl = 15176 * R_\lambda - 312.78$	0.758
$R_{[549-560]}^{-1} - R_{[750]}^{-1}$	$Chl = 15426 * R_\lambda - 35.154$	0.767
$R_{[710-718]}^{-1}$	$Chl = 27658 * R_\lambda - 486.54$	0.775
$R_{[710-718]}^{-1} - R_{[750]}^{-1}$	$Chl = 28484 * R_\lambda - 21.317$	0.795
SR, $R_{780} / R_{685}$	$Chl = 253.1 * R_\lambda - 709.67$	0.511
NDVI, $R_{[780-685]} / R_{[780+685]}$	$Chl = 3768.9 * R_\lambda - 1962.4$	0.528
GNDVI, $R_{[780-550]} / R_{[780+550]}$	$Chl = 2239.8 * R_\lambda - 490.8$	0.945

$\dagger$ Reflectance index.



**Figure 7.** Relationship of total chlorophyll content with reflectance indices: simple ratio (A) normalized difference vegetation index (B) and green normalized difference vegetation index (C).

**Table 3.** Relationship between predicted and measured total leaf chlorophyll (Chl) content using calibrated models developed from an independent data set.

$^{\dagger}R_{\lambda}$	Model	RMSE (mg m <sup>-2</sup> )	SE
$R_{[(549-560)]}^{-1}$	$Chl_{pred} = 0.7349 * R_{\lambda} + 107.91$	53.0	48.7
$(R_{[(549-560)]}^{-1} - R_{[(750)]}^{-1})$	$Chl_{pred} = 0.7197 * R_{\lambda} + 112.21$	53.6	48.8
$R_{[(710-718)]}^{-1}$	$Chl_{pred} = 0.7023 * R_{\lambda} + 124.66$	52.4	44.4
$(R_{[(710-718)]}^{-1} - R_{[(750)]}^{-1})$	$Chl_{pred} = 0.6728 * R_{\lambda} + 133.28$	54.0	45.1
SR $[R_{780}/R_{685}]$	$Chl_{pred} = 0.3099 * R_{\lambda} + 262.59$	92.3	67.4
NDVI $[(R_{780} - R_{685})/(R_{780} + R_{685})]$	$Chl_{pred} = 0.3134 * R_{\lambda} + 259.63$	90.4	65.8
GNDVI $[(R_{780} - R_{550})/(R_{780} + R_{550})]$	$Chl_{pred} = 0.7237 * R_{\lambda} + 101.18$	56.0	52.6

$^{\dagger}$ Reflectance index.

reflectance in the red range were sensitive only to low chlorophyll and not sensitive to moderate to high chlorophyll.

Due to the moderate to high chlorophyll of the data set, reflectance in the red spectral region had a low relationship to chlorophyll, and consequently it was not surprising that both NDVI and SR were poorly related to chlorophyll in this study. The calibrated models were used to predict chlorophyll from an independent second data set collected from the same study. Reciprocal reflectance values in the independent data set were used in the indices of the calibrated model to estimate chlorophyll. The estimated chlorophyll was then compared with measured chlorophyll content. Table 3 shows the RMSE and standard error of estimation between the predicted and the measured chlorophyll. The proposed models performed well in predicting chlorophyll, with RMSE ranging from 52 to 56 mg m<sup>-2</sup> (Table 3). As expected both NDVI and SR did poorly with very high RMSE while GNDVI performed better.

## Conclusion

With adequate plant water, both SPAD values and chlorophyll content had linear relationships with N application rate. Under water stress conditions, there was the tendency for SPAD values to under-estimate the N status of the plant. Leaf reflectance of grain sorghum was reduced by both N and water stress. Reciprocal reflectance of 549 to 560 nm,  $R_{[(549-560)]}^{-1}$  with the peak at 550 nm,  $R_{[(550)]}^{-1}$  and 710 to 718 nm,  $R_{[(710-718)]}^{-1}$  with peak at 718 nm,  $R_{[(718)]}^{-1}$  minus reciprocal reflectance in the NIR,  $R_{[(750)]}^{-1}$  had linear relationships with chlorophyll. The models calibrated in this study predicted leaf total chlorophyll with less RMSE than SR, NDVI and GNDVI.

## Conflict of interests

The authors have not declared any conflict of interest.

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## Full Length Research Paper

# Reactions of improved faba bean varieties to chocolate spot (*Botrytis fabae* Sard.) epidemics across contrasting altitudes in southwest Ethiopia

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Chocolate spot (*Botrytis fabae* Sard.) is one of the major diseases menacing faba bean (*Vicia faba* L.) production and restraining its productivity in Ethiopia and other African countries. The yield losses reach up to 100% on susceptible cultivars. Although a number of faba bean varieties with good yield potential have been released, their reaction to this major disease and yield performance are little understood in southwest Ethiopia. Thus, chocolate spot epidemics on 13 improved faba bean varieties were studied under natural infections at three sites varying in altitudes from 1805 to 2660 m in the Dawuro zone of southwest Ethiopia. The field experiments consisted of 14 treatments (13 varieties and a local cultivar) are laid out in a randomized complete block design (RCBD) with three replications (40 plants/plot) during the 2011/2012 crop season. The onset and progress of chocolate spot severity was assessed (with a 1-9 scale) every seven days until the epidemic attained peak and then grain yield and 100-seed weight were recorded and statistically analysed. The varieties varied significantly ( $P < 0.001$ ) in disease severity index, AUDPC and infection rates ( $r$ ) values, grain yield and 100-seed weight. CS20DK, Degaga, Nc-58, Bulga-70, Tesfa and Kasa exhibited high to moderate resistance to chocolate spot with consistently slow progression and terminal disease severity, AUDPC- and  $r$ -values at all testing sites. The yield performance of CS20DK and Degaga were also superior at Tocha and Turi while Nc-58 (2027 kg/ha) and Moti (1973 kg/ha) that showed susceptible reaction gave good yield only at Tocha. This study indicated that improved faba bean varieties reacted differently to chocolate spot infection and yield potentials across varying localities, and thus better performing varieties such as CS20DK, Degaga and Nc-58 are recommended for faba bean production in southwest Ethiopia.

**Key words:** *Botrytis fabae*, evaluation, improved varieties, *Vicia faba*.

## INTRODUCTION

Faba bean (*Vicia faba* L.) has been grown in the highlands of Ethiopia between 1800 and 3000 m above

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**Table 1.** Altitude and soil<sup>a</sup> characteristics of the experimental sites in Dawuro, southwest Ethiopia.

Study site	Altitude (m)	pH	CEC (Meq/gm)	C	OM	Total N	Av. P	Texture			
								Sand	Clay	Silt	Class
Tocha	2660	5.4	0.1	1.37	2.35	74.2	0.12	28	18	54	Silty loam
Mari	2445	5.2	0.06	0.78	1.34	52.8	0.07	36	22	42	Loam
Turi	1805	5.7	0.06	1.13	1.95	46.2	0.10	24	26	50	Silty loam

<sup>a</sup> Soil samples taken at 20 cm depth and analyzed at Hawasa Soil Laboratory, Ethiopia. CEC, C, OM, Av. P refers to cation exchange capacity, carbon, organic matter and available phosphorous, respectively.

sea level (a.s.l.) for many decades and the country is now considered as one of the centers of secondary diversity for the crop (Agegnehu et al., 2006). The crop occupies the largest areas among legumes and the total area under cultivation is estimated to be about 512,067 ha from which 200,000 metric tonnes of grain yield is harvested (FAOSTAT, 2010). The primary producers of pulses are small-scale farmers with small and dispersed plots under rain-fed conditions with substantially lower yields of less than 0.9 t/ha as compared to the improved faba bean varieties and international yields.

Chocolate spot (*Botrytis fabae* Sard.) is the major fungal disease hampering faba bean production in Ethiopia (Dereje and Yaynu, 2001; Sahile et al., 2010). It is a highly prevalent and destructive disease, causing yield loss up to 61% on a susceptible and 34% on tolerant faba bean genotypes in the central highlands (Dereje and Yaynu, 2001). Sahile et al. (2010) reported even higher losses of 67.5% in the unsprayed faba bean plots in northwest Ethiopia.

The extent of chocolate spot damage and genetic diversity of *B. fabae* has been less understood in southwestern parts of Ethiopia although many research activities, including development and release of improved faba bean varieties, have been undertaken in the cereal-

pulse based farming system of the central highlands of the country. To-date, more than 20 improved varieties with grain yield varying between 1.8 to 4.0 t/ha have been released for production. The newly developed varieties include CS20DK, Kusse-2-27-33, Bulga 70, NC-58, Kassa, Tesfa and Degaga (MoAR, 2009). However, the adoption of these improved faba bean varieties has been challenged by many biotic and abiotic factors, in particular by chocolate spot (*B. fabae*) infections. Besides the considerable differences in soil and weather conditions across faba bean belts, there exists wide variation in pathogenicity among *B. fabae* populations collected from diverse regions (Dereje, 1996). Sahile et al. (2012) indicated differences in virulence among 76 *B. fabae* isolates collected from faba bean fields in northern Ethiopia.

The production of the crop is enormously declining as the local faba bean cultivars are entirely attacked by chocolate spot and other diseases in southwest Ethiopia. The deployment of resistant varieties to this calamitous disease is one of the management approaches and in order to identify the resistant varieties, the test genotype has to be assessed under naturally occurring heavy disease zones (hot spot) and through artificial infection (Bond et al., 1994; Villegas-

Fernandez et al., 2009). In this study, the epidemics of chocolate spot (*B. fabae*) on 13 improved faba bean varieties was carried out under field conditions across three altitudinal gradients. The performance of the improved varieties *vis-vis* the respective local cultivars/landraces in reaction to the disease and their yield potential are discussed.

## MATERIALS AND METHODS

### Description of the study sites

Chocolate spot epidemics were studied on thirteen released faba bean varieties and a local cultivar under field conditions in three districts representing potential faba bean producing areas of the Dawuro zone in southwest Ethiopia during the 2011/2012 crop season. The sites were Tocha, Mari and Turi situated at varying altitudes between 1800 and 2660 m a.s.l (Table 1) and characterized by wet and humid climatic conditions where chocolate spot is known to be consistently prevalent and severe on local cultivars.

### Faba bean varieties

Seeds of thirteen improved faba bean varieties with relatively high yield (1.5 – 4.0 t/ha) that had been released for production between 1978 and 2010 (MoARD, 2009; EARO, 2010) were obtained from Holleta Agricultural Research Center, Ethiopia. A 'local cultivar' commonly

**Table 2.** Agronomic characteristics of released faba bean varieties used in the study.

Faba bean variety	Pedigree name	Production domain (m)	Maturity days	Yield (qt/ha)	Year of release
Bulga-70	Coll 111/77	2300-3000	143-150	15-35	1994
CS20DK	CS20DK	2300-3000	145-160	20-40	1977
Degaga	R-878-3	1800-3000	116-135	20-45	2002
Gebelcho	Tesfa x ILB4726	1800-3000	103-167	20-30	2006
Hachalu	EH960091-1	1900-2800	122-156	24-35	2010
Kasa	NA	1900-2300	120-135	25-40	1980
Kuse	Kuse 2-27-33	2300-3000	135-150	15-25	1979
Mesay	74TA12050 x 74TA236	1800-2400	120-130	20-45	1995
Moti	ILB4432 x Kuse 2-27-33	1800-3000	108-165	23-35	2006
NC-58	NC-58	1900-2300	118-132	15-35	1978
Tesfa	75TA26026-1-2	1800-2400	125-135	20-45	1995
Tumsa	EH9965-3	2050-2800	121-176	20-38	2010
Walki	Bulga-70 x ILB4615	1800-2800	133-146	20-42	2007
Local <sup>a</sup>	-	-	-	-	-

<sup>a</sup> Local cultivars grown by the farmers at respective study area. Source: MoARD 2009, EARO 2010.

grown by farmers in the respective study areas was included as a susceptibility check (Table 2).

#### Land preparation, planting and field management

The faba bean varieties were sown (2 seeds /hill) on properly prepared land at a recommended population density of 250,000 plants/ ha at 10 cm (between plants) x 40 cm (between rows) (EARO, 2010) in July 2011. In order to ensure the disease occurrence and enhance the natural infections, a row of a chocolate spot susceptible local cultivar was planted between each plot. This practice is reported to induce early disease development on local checks which can later serve to spread the pathogen spores to adjacent test entries (ICARDA, 1986; Bouhassen et al., 2004). The experimental plots were arranged in a randomized complete block design with three replications while each plot had four rows with 10 plants/row. The recommended agronomic practices like weeding were uniformly applied to each plot throughout the study period. Faba bean plants in the central two rows were used for

disease assessment, yield and yield related measurements.

#### Disease assessment

Chocolate spot severity was recorded on each of eight sample plants from middle two rows in each plot, using 1 – 9 scale; where, 1 = no lesions or covering up to 1% of leaf surface; 3 = lesions covering 1 – 2 % of leaf surface; 5 = lesions common (3 – 5 mm in diameter), covering 2 – 5% of leaf surface; 7 = lesions that cover 5 – 10 % of leaf surface; 9 = extensive lesions, covering more than 10% of the leaf surface (ICARDA, 1986; Hanounik, 1986). The disease was scored at weekly intervals starting from the first chocolate spot symptom appearance and continued until the final podding stage when the disease attained maximum (Sahile et al., 2008; Villegas–Fernandez et al., 2012). Finally, the scores were converted into disease severity index (DSI) for the analysis (Hanounik, 1986; Ayman et al., 2009; Abo-Hegazy et al., 2012). Based on DSI values, the response of tested varieties were classified

into six reaction groups following Abo-Hegazy et al. (2012); where 0 – 2% is highly resistant (HR), >2 – 15% resistant (R), >15 – 40% moderately resistant (MR), >40 – 60% is moderately susceptible (MS) and >60 – 80% is susceptible (S) >80 – 100% highly susceptible (HS).

#### Yield data

In addition to disease data, the varieties were evaluated for yield and related components. Grain yield (gm/plot) and 100- seeds weight were recorded from plants in the central rows of each plot at harvest, and finally the yield was converted to kg/ha before statistical analysis.

#### Disease epidemics analysis

The progress of chocolate spot was plotted over time using mean severity indices for each faba bean variety at each location, and the DSI values were also used for the two important epidemiological analyses, namely, area under

**Table 3.** Analysis of variance of disease severity index (DSI) on 13 faba bean varieties and a local cultivar at all assessment dates under field conditions at Tocha, Mari and Turi in Dawuro southwest Ethiopia during 2011/2012 cropping season.

Location	Source of variation	DF	Disease severity index (days after planting)									
			35	42	49	56	63	70	77	84	91	98
Tocha	Variety	13	17.9**	25.9**	34.5**	37.1**	27.7**	60.2**	72.0**	100.5**	128.4**	128.8**
	Block	2	0.8 <sup>ns</sup>	1.1 <sup>ns</sup>	7.6 <sup>ns</sup>	3.7 <sup>ns</sup>	0.2 <sup>ns</sup>	3.0 <sup>ns</sup>	4.5 <sup>ns</sup>	0.75 <sup>ns</sup>	0.5 <sup>ns</sup>	2.3 <sup>ns</sup>
	Residue	26	1.2	2.2	3.2	2.7	1.2	1.8	2.3	1.2	0.3	1.1
Mari	Variety	13	6.6*	9.4**	5.9*	8.6**	33.8**	57.6**	113.9**	153.2**	182.4**	189.9**
	Block	2	1.7 <sup>ns</sup>	0.6 <sup>ns</sup>	3.5 <sup>ns</sup>	2.2 <sup>ns</sup>	6.7 <sup>ns</sup>	6.7 <sup>ns</sup>	1.7 <sup>ns</sup>	0.3 <sup>ns</sup>	2.1 <sup>ns</sup>	0.2 <sup>ns</sup>
	Residue	26	1.9	1.2	1.9	1.9	4.1	3.12	2.3	1.3	2.4	1.2
Turi	Variety	13	- <sup>a</sup>	0.9*	5.8**	7.4**	10.7**	12.9**	8.9**	6.7**	9.3**	6.2**
	Block	2	-	0.6 <sup>ns</sup>	3.9*	1.7 <sup>ns</sup>	2.9 <sup>ns</sup>	2.3 <sup>ns</sup>	1.3 <sup>ns</sup>	0.2 <sup>ns</sup>	0.7 <sup>ns</sup>	1.0 <sup>ns</sup>
	Residue	26	-	1.2	0.9	1.2	1.6	1.6	1.5	0.9	1.1	1.3

<sup>a</sup> no disease record. DF refers to degrees of freedom, \* and \*\* indicate levels of significance at  $P < 0.05$  and  $P < 0.01$  levels, respectively, ns stands for non significant.

disease progress curve (AUDPC) and apparent infection rate ( $r$ ). The AUDPC values (%-day) were calculated for each variety according to the mid-point rule formula (Berger, 1981; Campbell and Madden, 1990).

$$AUDPC = \sum_{i=1}^{n-1} \left( \frac{Y_i + (Y_{i+1})}{2} \right) T(i+1) - T_i$$

Where  $Y_i$  is the disease severity index (DSI) of chocolate spot at  $i^{\text{th}}$  assessment date,  $T_i$  is the time of the  $i^{\text{th}}$  assessment in days from the first assessment date and  $n$  is the total number of disease assessments. Because severity was in percentage and time in days, AUDPC was expressed in proportion days.

The apparent infection rate, expressed in disease units per day, was calculated first by converting DSI values to a proportion on a scale of 1 to 9 and then transformed to logistic model ( $\ln \left[ \frac{Y}{1-Y} \right]$ ) (Van der Plank, 1963) where  $Y$  and  $1-Y$  represent the proportion of infected plants and the proportion of healthy plants remaining in the plot, respectively. The transformed values ( $y$ ) were regressed

against time ( $x$ ) to determine the model (Campbell and Madden, 1990).

#### Statistical analysis

The disease severity values (DSI, AUDPC,  $r$ ), seed yield (kg/ha) and 100-seed weight (gm/plot) were analysed and treatment means were compared with SAS software version 9.2 (SAS Institute, 2008). The relationship among the disease parameters with seed yield was tested using simple correlation-regression analysis.

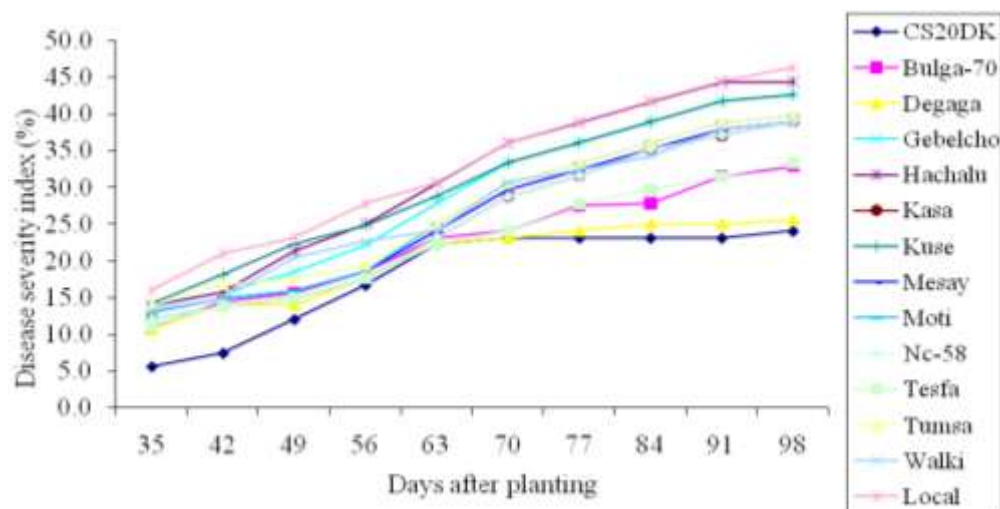
## RESULTS

### Chocolate spot appearance and epidemics

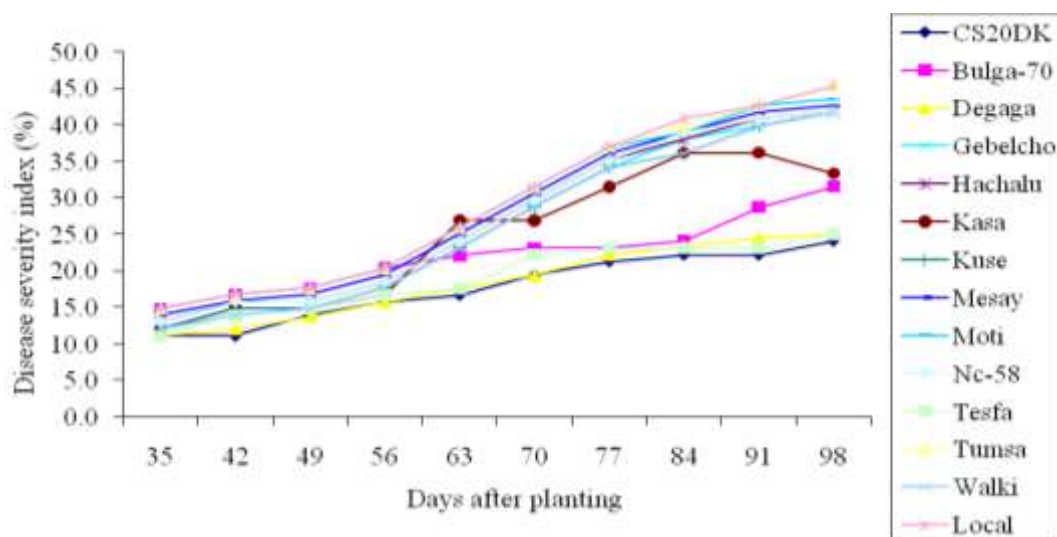
The earliest characteristic symptoms of chocolate spot, which are small discrete reddish-brown sunken lesions on the upper side of the leaves,

appeared first on the susceptible local faba bean cultivar in the spreader rows at the Tocha and Mari experimental sites both situated at higher altitudes (>2445 m a.s.l.). Subsequently, the disease developed on some of the improved varieties such as Hachalu, Messay and Walki, 20 days after planting (DAP) at the same sites while it occurred a week later (27 DAP) at Turi (1805 m a.s.l.).

The disease severity indices analysis showed significant ( $P < 0.01$ ) differences among the 13 faba bean varieties at all assessment dates between 35 and 98 DAP in the three sites (Table 3). Chocolate spot severity was significantly lower ( $< 23\%$ ) on CS20DK and Degaga followed by Tesfa and Bulga-70 while the local cultivar and most of the improved varieties Hachalu, Kuse and Gebelcho showed higher disease infections ranging from 42 to 45% at Tocha particularly 63



**Figure 1.** Chocolate spot epidemics on 13 improved faba bean varieties and a local cultivar at Tocha (2660 m.a.s.l.), in Dawuro southwest Ethiopia during 2011/2012 cropping season.



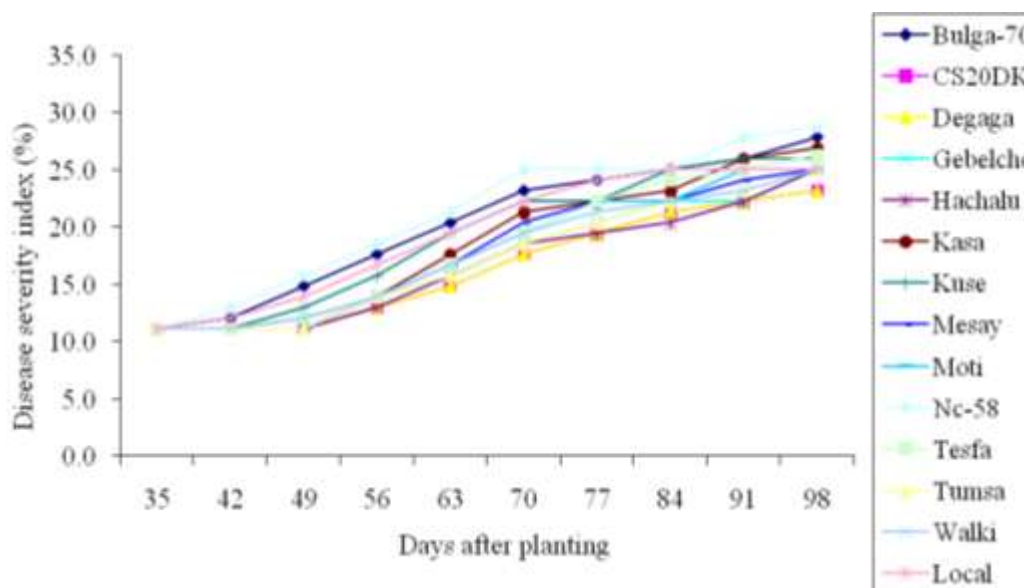
**Figure 2.** Chocolate spot epidemics on 13 improved faba bean varieties and a local cultivar at Mari (2445 m.a.s.l.) in Dawuro southwest Ethiopia during 2011/2012 cropping season. Local cultivar refers to faba bean landraces commonly grown by most farmers.

DAP (Figure 1). Similarly, there were distinct differences among the faba bean varieties in the disease progression at Mari where the severity levels were more pronounced on all plants of the local cultivar; Moti, Messay and Nc-58 during all the successive assessments 56 DAP as opposed to that of CS20DK, Tesfa and Degaga (Figure 2). In general, the final chocolate spot severity was significantly different ( $P < 0.05$ ) among the varieties at Turi (Figure 3) though the infection values were lower than that of Mari and Tocha.

The highest chocolate spot infections on the local

cultivars were 46.3 and 45.0% at Tocha and Mari, respectively, as opposed to the lowest mean severity of 24.1% on CS20DK and 25% on Degaga at both sites (2445 to 2660 m a.s.l.) (Table 4). Hachalu and Kuse varieties had relatively high infection values of 44.4 and 42.6% similar to the local susceptible check. Bulga-70 and Tesfa had moderate infections that varied from 31.5 to 32.8 and 25.0 to 33.3% at Tocha and Mari, respectively (Table 4). Nevertheless, maximum disease severity of 28.7% was recorded on Nc-58 and it was not significantly different from other varieties except from that





**Figure 3.** Chocolate spot epidemics on 13 improved faba bean varieties and a local cultivar at Turi (1805 m.a.s.), Dawuro in southwest Ethiopia during 2011/2012 cropping season.

**Table 4.** Terminal chocolate spot severity indices<sup>a</sup> (%) on 13 improved faba bean varieties and local checks at three varying sites in Dawuro southwest Ethiopia during 2011/2012 main crop season.

Faba bean variety	Tocha (2660 m a.s.l),	Mari (2445 m a.s.l)	Turi (1805 m a.s.l)
Bulga-70	32.8 <sup>e</sup>	31.5 <sup>c</sup>	24.1 <sup>bc</sup>
CS20DK	24.1 <sup>f</sup>	24.1 <sup>d</sup>	23.1 <sup>c</sup>
Degaga	25.5 <sup>f</sup>	24.7 <sup>d</sup>	23.0 <sup>c</sup>
Gebelcho	42.6 <sup>bc</sup>	41.7 <sup>b</sup>	25.0 <sup>bc</sup>
Hachalu	44.4 <sup>ab</sup>	41.7 <sup>b</sup>	25.0 <sup>bc</sup>
Kasa	38.9 <sup>d</sup>	33.3 <sup>c</sup>	27.0 <sup>ab</sup>
Kuse	42.6 <sup>bc</sup>	42.0 <sup>b</sup>	26.0 <sup>a-c</sup>
Mesay	38.9 <sup>d</sup>	43.0 <sup>ab</sup>	25.0 <sup>bc</sup>
Moti	39.8 <sup>cd</sup>	44.0 <sup>ab</sup>	25.0 <sup>bc</sup>
Nc-58	38.9 <sup>d</sup>	42.0 <sup>b</sup>	28.7 <sup>a</sup>
Tesfa	33.3 <sup>e</sup>	25.0 <sup>d</sup>	26.0 <sup>a-c</sup>
Tumsa	39.8 <sup>cd</sup>	45.0 <sup>a</sup>	25.0 <sup>bc</sup>
Walki	38.9 <sup>d</sup>	42.0 <sup>b</sup>	25.0 <sup>bc</sup>
Local	46.3 <sup>a</sup>	45.0 <sup>a</sup>	25.0 <sup>bc</sup>
Mean	37.6	37.4	25.2
SD (±)	6.5	7.8	1.7
CV (%)	2.8	2.9	4.5
P-value	<0.0001	<0.0001	<0.0001

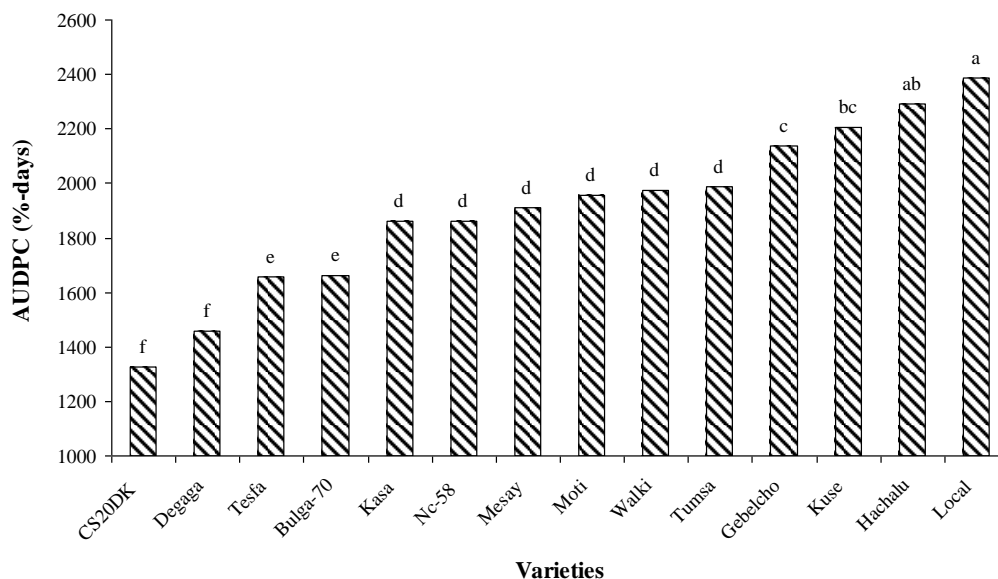
<sup>a</sup> DSI = mean disease severity index assessed at 98 days after planting (DAP). Means with the same letter(s) within each columns are not significantly different ( $P < 0.05$ ) based on Turkey's Studentized Range Test. Faba bean varieties with values >15 - 40 are considered moderately resistant while values >40 - 60 moderately susceptible (Abo-Hegazy et al., 2012).

of CS20DK and Degaga in the field at Turi (Table 4).

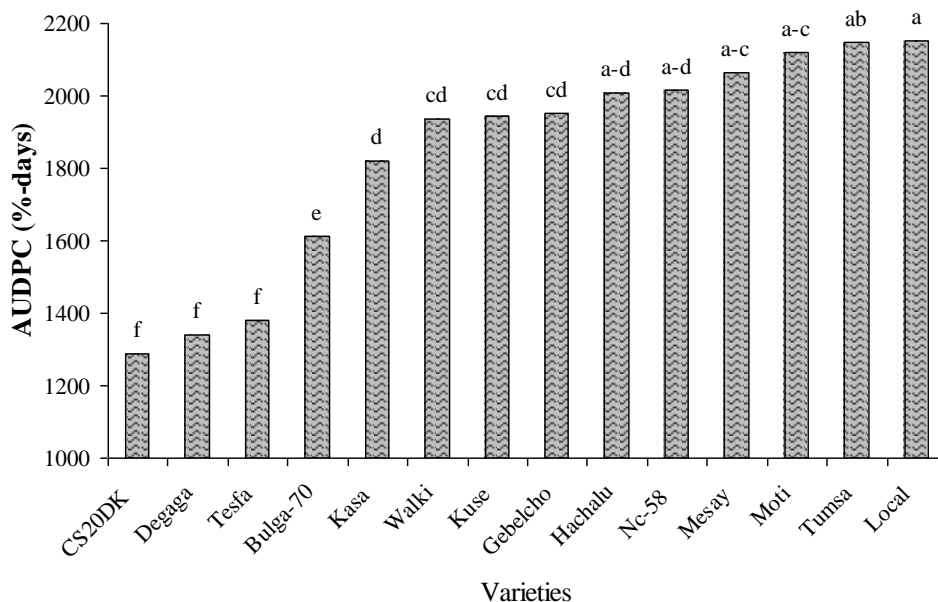
Accordingly, the four faba bean varieties namely CS20DK, Degaga, Bulga-70 and Tesfa expressed relatively moderate to high resistance to chocolate spot at Tocha and Mari under heavy disease pressure.

#### Area under disease progress curve (AUDPC) of chocolate spot

The area under disease progress curve (AUDPC) values for chocolate spot was significantly ( $P < 0.01$ ) different



**Figure 4.** The area under disease progress curve (AUDPC) values on 13 improved faba bean varieties and a local cultivar at Tocha during 2011/2012 cropping season. Bars with the same letter(s) are not significantly different ( $P < 0.05$ ) from each other.



**Figure 5.** The area under disease progress curve (AUDPC) values on 13 improved faba bean varieties and a local cultivar at Mari during 2011/2012 cropping season. Bars with the same letter(s) are not significantly different ( $P < 0.05$ ) from each other.

among the faba bean varieties tested at all locations. The overall AUDPC values ranged from 1289 to 2387 proportion - days for CS20DK and the local cultivar at Tocha and Mari (Figures 4 and 5). Faba bean varieties CS20DK and Degaga followed by Bulga-70 and Tesfa had low disease accumulation revealed by significantly ( $P < 0.05$ ) least mean AUDPC values in contrast to Hachalu,

Gebelcho, Tumsa and the local susceptible checks at both sites (Figures 4 and 5).

#### Chocolate spot infection rates

The logistic model that provided the best fit and

**Table 5.** Chocolate spot infection rates on 13 improved faba bean varieties and a local cultivar at Tocha, Mari and Turi sites in Dawuro southwest Ethiopia during 2011/2012 crop season.

Faba bean variety	Tocha				Mari				Turi			
	<sup>1</sup> <i>r</i>	<sup>2</sup> <i>S</i>	<sup>3</sup> <i>l</i>	<sup>4</sup> <i>R</i> <sup>2</sup>	<i>r</i>	<i>S</i>	<i>l</i>	<i>R</i> <sup>2</sup>	<i>r</i>	<i>S</i>	<i>l</i>	<i>R</i> <sup>2</sup>
Bulga-70	0.151 <sup>b</sup>	0.47	-2.11	93.8	0.131 <sup>bc</sup>	0.32	-1.82	87.6	0.122 <sup>def</sup>	0.39	-2.22	92.1
Cs-20Dk	0.181 <sup>ab</sup>	0.64	-2.62	73.8	0.110 <sup>c</sup>	0.34	-2.18	87.6	0.118 <sup>f</sup>	0.37	-2.31	93.1
Degaga	0.118 <sup>c</sup>	0.39	-2.07	83.9	0.118 <sup>c</sup>	0.37	-2.18	87.6	0.118 <sup>f</sup>	0.37	-2.31	93.6
Gebelcho	0.185 <sup>a</sup>	0.57	-1.97	96.3	0.207 <sup>a</sup>	0.64	-2.25	97.1	0.124 <sup>c-f</sup>	0.39	-2.30	94.7
Hachalu	0.192 <sup>a</sup>	0.60	-1.91	94.8	0.195 <sup>a</sup>	0.60	-2.13	96.7	0.122 <sup>ef</sup>	0.38	-2.32	93.1
Kasa	0.189 <sup>a</sup>	0.58	-2.20	96.6	0.170 <sup>ab</sup>	0.55	-2.11	86.6	0.139 <sup>a</sup>	0.44	-2.30	93.4
Kuse	0.167 <sup>ab</sup>	0.52	-1.81	95.4	0.175 <sup>a</sup>	0.62	-2.25	97.3	0.134 <sup>a-c</sup>	0.42	-2.23	92.8
Messay	0.181 <sup>ab</sup>	0.56	-2.11	96.4	0.170 <sup>ab</sup>	0.59	-2.06	96.6	0.131 <sup>a-e</sup>	0.41	-2.30	93.2
Moti	0.186 <sup>a</sup>	0.58	-2.13	95.9	0.186 <sup>a</sup>	0.57	-1.98	96.5	0.133 <sup>a-d</sup>	0.41	-2.31	94.7
Nc-58	0.189 <sup>a</sup>	0.58	-2.20	96.6	0.196 <sup>a</sup>	0.61	-2.13	96.5	0.131 <sup>a-e</sup>	0.41	-2.09	91.8
Tesfa	0.187 <sup>a</sup>	0.43	-2.25	84.9	0.104 <sup>c</sup>	0.34	-2.06	87.1	0.136 <sup>ab</sup>	0.42	-2.31	95.5
Tumsa	0.171 <sup>ab</sup>	0.52	-1.98	96.8	0.191 <sup>a</sup>	0.59	-2.00	97.1	0.126 <sup>b-f</sup>	0.39	-2.31	95.0
Walki	0.165 <sup>ab</sup>	0.52	-1.96	92.9	0.178 <sup>a</sup>	0.63	-2.27	97.3	0.124 <sup>c-f</sup>	0.39	-2.27	94.9
Local	0.167 <sup>ab</sup>	0.51	-1.69	96.0	0.192 <sup>a</sup>	0.59	-2.00	96.7	0.123 <sup>c-f</sup>	0.39	-2.14	91.2
Mean	0.173				0.166				0.127			
CV	10.7				15.3				5.15			
SD	0.025				0.034				0.009			
P-value	0.0031				<0.0001				0.0113			

<sup>1</sup>*r* =infection rate; <sup>2</sup>*S*=standard error of infection rate; <sup>3</sup>*l*=intercept; <sup>4</sup>*R*<sup>2</sup>=coefficient of determination for the logistic model (%). Intercepts and infection progress rates represent the equation of the line. Means in a column with the same letter(s) are not significantly ( $P < 0.05$ ) different from each other.

the mean apparent infection rate (*r*) of chocolate spot varied significantly ( $P < 0.05$ ) among the varieties at the three sites (Table 5). Low infection rates of 0.110, 0.118 and 0.104 disease units per day were computed for Degaga, CS20Dk and Tesfa, respectively at Mari; and for the former two varieties at Turi (0.118 disease units per day). Degaga showed consistently slower infection rate across the three sites while CS20DK has shown slightly varied response, faster disease development at Tocha but slower at Mari and Turi

(Table 5). The regression equations with coefficient of determinations (*R*<sup>2</sup>) ranging from 73.8 to 97.3% were calculated when the severity indices were regressed over time in days after planting for all faba bean varieties (Table 5).

#### Grain yield and 100-seed weight of faba bean varieties

There were highly significant ( $P < 0.01$ ) differences

among the tested faba bean varieties for both grain yield and 100-seed weight at all experimental sites (Table 6). The highest seed yield of 2100 and 2027 kg/ha was harvested from CS20Dk and Nc-58, respectively; followed by Moti (1973 kg/ha) and Degaga (1910 kg/ha) at Tocha. Bulga-70 (1500 kg/ha) and Kasa (1520 kg/ha) produced significantly ( $P < 0.05$ ) lower yield than the local cultivar (1873 kg/ha) (Table 6). Among varieties, Hachalu (99.1 g), Moti (91.3 g), Gebelcho (86.3 g) and Tumsa (84.5 g) were

**Table 6.** Performance of faba bean varieties in grain yield (kg/ha) and hundred seed weight (g) at Tocha, Mari and Turi experimental sites in Dawuro southwest Ethiopia during 2011/2012 crop season.

Faba bean variety	Grain yield (kg/ha)			100- seed weight (g)		
	Tocha	Mari	Turi	Tocha	Mari	Turi
Bulga-70	1500 <sup>g</sup>	813 <sup>bc</sup>	963 <sup>bc</sup>	44.1 <sup>gh</sup>	39.3 <sup>g</sup>	42.3 <sup>gh</sup>
CS20Dk	2100 <sup>a</sup>	880 <sup>b</sup>	1043 <sup>ab</sup>	55.3 <sup>ef</sup>	57.7 <sup>e</sup>	49.0 <sup>f</sup>
Degaga	1910 <sup>b-d</sup>	1327 <sup>a</sup>	1221 <sup>a</sup>	62.4 <sup>d</sup>	58.7 <sup>e</sup>	53.7 <sup>e</sup>
Gebelcho	1810 <sup>c-e</sup>	803 <sup>bc</sup>	1013 <sup>bc</sup>	86.3 <sup>b</sup>	71.2 <sup>cd</sup>	77.1 <sup>b</sup>
Hachalu	1890 <sup>b-e</sup>	840 <sup>bc</sup>	937 <sup>bc</sup>	99.1 <sup>a</sup>	95.7 <sup>a</sup>	82.8 <sup>a</sup>
Kasa	1520 <sup>g</sup>	813 <sup>bc</sup>	1027 <sup>bc</sup>	58.5 <sup>de</sup>	54.0 <sup>e</sup>	72.3 <sup>c</sup>
Kuse	1570 <sup>fg</sup>	863 <sup>bc</sup>	977 <sup>bc</sup>	38.6 <sup>h</sup>	35.7 <sup>g</sup>	28.6 <sup>i</sup>
Messay	1305 <sup>h</sup>	737 <sup>c</sup>	1050 <sup>ab</sup>	50.7 <sup>fg</sup>	47.2 <sup>f</sup>	44.5 <sup>g</sup>
Moti	1973 <sup>a-c</sup>	760 <sup>bc</sup>	970 <sup>bc</sup>	91.3 <sup>b</sup>	83.7 <sup>b</sup>	84.0 <sup>a</sup>
Nc-58	2027 <sup>ab</sup>	870 <sup>b</sup>	993 <sup>bc</sup>	56.7 <sup>d-f</sup>	53.3 <sup>ef</sup>	48.0 <sup>f</sup>
Tesfa	1840 <sup>c-e</sup>	790 <sup>bc</sup>	903 <sup>bc</sup>	46.7 <sup>g</sup>	38.3 <sup>g</sup>	39.6 <sup>g</sup>
Tumsa	1773 <sup>de</sup>	833 <sup>bc</sup>	860 <sup>cd</sup>	84.5 <sup>b</sup>	73.7 <sup>c</sup>	66.1 <sup>d</sup>
Walki	1727 <sup>ef</sup>	820 <sup>bc</sup>	873 <sup>b-d</sup>	44.1 <sup>c</sup>	65.3 <sup>d</sup>	69.8 <sup>c</sup>
Local cultivar	1873 <sup>b-e</sup>	533 <sup>d</sup>	697 <sup>d</sup>	38.6 <sup>h</sup>	36.3 <sup>g</sup>	40.8 <sup>h</sup>
Mean	1772.7	834.5	996.2	63.3	57.9	57.0
SD (±)	225	165.3	125.1	19.8	18.1	17.6
CV (%)	3.8	5.1	6.1	3.4	3.5	1.8
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

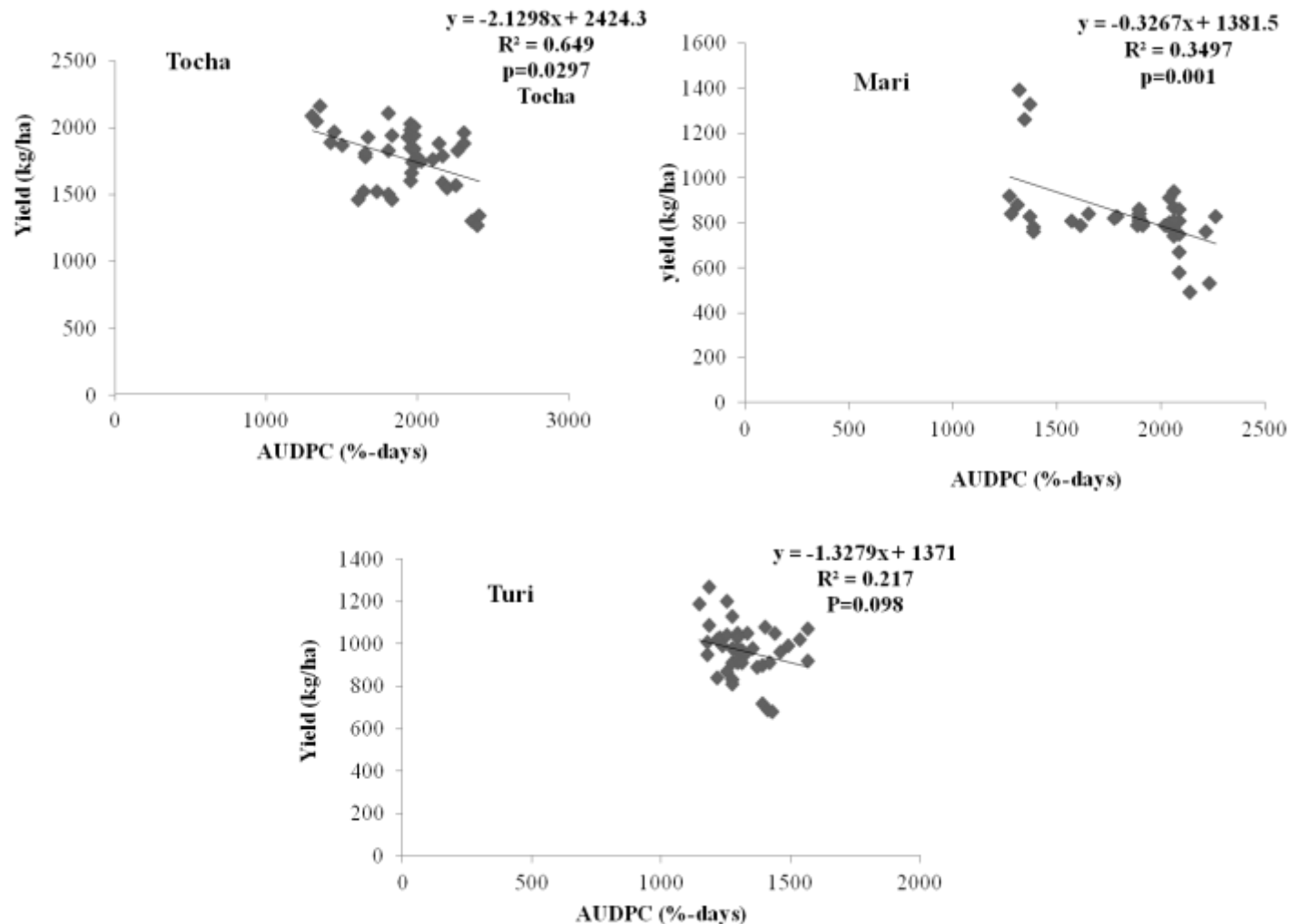
Means in a column with the same letter(s) are not significantly ( $P < 0.05$ ) different from each other.

significantly ( $P < 0.05$ ) superior in 100-seed weight to Bulga-70, Kuse and the local cultivar at Tocha (Table 6). Similarly heavier seeds were recorded from Hachalu (95.6 g), Moti (83.7 g), Tumsa (73.7 g) and Gebelcho (71.2 g) at Mari. Moti (84 g), Hachalu (82.8 g) and Gebelcho (77.1 g) were the leading varieties at Turi (Table 6).

#### Relationship between chocolate spot severity and yield

There were inverse relationship between chocolate spot severity recorded throughout the assessment period and faba bean yield with negative correlation values ranging from -0.592 to

-0.324, and from -0.563 to -0.394 at Tocha and Mari, respectively (Table 7). The association was negative but not strong for all except the disease severity level (-0.335) scored 84 DAP at Turi (Table 7). In addition, the linear regression of AUDPC better described the impact of disease severity on grain yield at Tocha and Mari, and that



**Figure 6.** Linear regression model relating chocolate spot progress against yield on 14 faba bean varieties at Tocha, Mari and Turi experimental sites during 2011/2012 cropping season.

about 21.7 to 60.8% grain yield was accounted to chocolate spot infections while the estimated slope of regression lines were -2.1298, -0.3267, and -1.3279 for seed yield at Tocha, Mari, and Turi, respectively (Figure 6).

## DISCUSSION

Host resistance to chocolate spot pathogen (*B. fabae*) is the major element that could slow the disease epidemics and lead to successful control. Sources of resistance for the disease have been identified from introductions of ICARDA to Ethiopia and most of them have been nationally or regionally released for different recommendation domains (Mussa et al., 2008). In the present study, 13 improved faba bean varieties were evaluated for their reactions to chocolate spot epidemics under natural conditions at three varying localities where

the crop is predominantly grown by small-scale farmers in Dawuro zone of southwest Ethiopia. The performances of these varieties were critically assessed for disease progress, infection rates and severity levels at varying growth stages of faba bean plants and finally for grain yield and hundred seed weight.

The result demonstrated that the tested faba bean varieties showed remarkable differences and CS20DK, Degaga, Tesfa and Bulga-70 were moderately resistant (MR) at Tocha and Mari exhibiting reduced infection rates and the lowest disease severity of less than 23 per cent. Whereas, most improved varieties, including Hachalu, Kuse and Gebelcho, were moderately susceptible with 42 to 45 percent severity, which was similar to the local cultivars at the same localities. The moderately resistant varieties limited pathogen spread effectively which slowed down the disease at different phases of progression as shown by relatively lower infection rates and AUDPC values.

**Table 7.** Coefficient of correlation between chocolate spot severity at varying growth stage and grain yield of fourteen faba bean varieties at three localities in Dawuro, southwest Ethiopia during 2011/2012 cropping season.

Days after planting	Tocha		Mari		Turi	
	Coefficient of correlation	P-value	Coefficient of correlation	P-value	Coefficient of correlation	P-value
35	-0.453	0.0026	-0.394	0.010	_a	_a
42	-0.592	0.0001	-0.524	0.000	-0.149	0.347
49	-0.505	0.0006	-0.418	0.006	-0.262	0.094
56	-0.491	0.001	-0.477	0.001	-0.261	0.095
63	-0.361	0.0189	-0.493	0.001	-0.295	0.058
70	-0.324	0.0364	-0.563	0.000	-0.245	0.118
77	-0.369	0.0163	-0.504	0.001	-0.268	0.086
84	-0.362	0.0185	-0.499	0.001	-0.335	0.030
91	-0.386	0.0116	-0.474	0.002	-0.166	0.293
98	-0.439	0.0037	-0.528	0.000	-0.153	0.333

<sup>a</sup> no disease occurrence.

Bouhassan et al. (2004) found nine highly resistant to chocolate spot out of 136 faba bean collections evaluated under field and laboratory conditions with low AUDPC scores, low AUDPC of lesion diameter and low spore production. El-Sayed et al. (2011) reported remarkable variations among five faba bean cultivars with higher disease infection in the first season (2007/2008) compared with the second (2008/09). Similar results were reported by Abo-Hegazy et al. (2012) who found significant variation among 13 faba bean genotypes in their response to *B. fabae* infections under field conditions. In general, moderate resistance to chocolate spot with partial dominance has been reported and the uses of moderately resistant cultivars are advised instead of depending solely on fungicides (Hanounik and Robertson, 1988; Bouhassan et al., 2004; Josefina, 2010).

With regards to grain yield, CS20DK, Nc-58, Degaga and Moti were top yielding varieties with

1973 to 2100 kg/ha though there was inconsistent performance that could be partly explained by differences in soil texture and fertility across the experimental sites. There was also a moisture shortage as rain stopped earlier during grain filling stage at Turi. It was indicated that faba beans are vulnerable to soil compaction and soil pH (Elliott and Whittington, 1978). However, Degaga had consistent yield potential at the three localities that perhaps attributed to its vigorous vegetative growth and robust root system to exploit the residual moisture available.

It can be concluded from these results that those faba bean varieties namely CS20DK and Nc-58 with moderate resistance to *B. fabae* infection and superior yield performance are recommended to be used in chocolate spot prone areas of southwest Ethiopia. In addition, Degaga also can be grown in areas with contrasting environments within the faba bean production domain.

### Conflict of Interests

The authors have not declared any conflict of interests.

### ACKNOWLEDGEMENTS

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*Full Length Research Paper*

# Assessing the effects of agroecology and conventional farming techniques on small-scale peasant farmers' crop yields in the Fako and Meme divisions of Cameroon

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Small-scale farming constitutes a very important segment of the food production chain in most third world countries. In Cameroon for example, they constitute about 70% of the agrarian population. This study aimed at verifying the effects of agroecology and conventional farming techniques on crop yields in four sites in the South West Region of Cameroon from small-scale farms. Data were obtained through the administration of 200 questionnaires and two focus group discussions (FGDs). The data were analyzed using frequencies, means, coefficient of correlation, coefficient of determination, and linear regression models. The FGDs were also analysed using context analysis. All the analyses were performed in SPSS version 20 and Wordstat 7 software. The results showed that both agroecology and conventional farming techniques are used in the study sites but agroecology techniques are more responsible for yield increases than conventional techniques as seen in correlations coefficients and regression outputs. The only exceptions in which conventional farming techniques contribute more to yields was under income levels and the number of family members that live and work on the farm. This was justified by the fact that conventional techniques often require higher income levels since they are often purchased.

**Key words:** Agroecology and conventional techniques, small-scale farmers, crop yields.

## INTRODUCTION

Agriculture is important to Cameroon's economy, because, it employs between 70 and 80% of the population, accounts for close to 50% of export earnings

and contributes 30% to the Gross Domestic Product (GDP) of the country (DSCN, 2002). A majority of the population involved in agriculture is dominated by small-

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scale farmers and their family members who constitute about 70% of the agricultural population (National Institute of Statistics, 2010).

Cameroon is currently experiencing declines or stagnation in food production (Epule and Bryant, 2015). Much of this decline or stagnation has been attributed to declines in soil nutrients (Gould et al., 1989; Nkamleu and Adesina, 2000; Kombiok et al., 2013), population growth and resultant expansion of agricultural land through deforestation (Palm et al., 2001; Epule and Bryant, 2015). As a result of these observations, a lot of efforts are being made to increase crop yields and reduce food insecurity. Much of these efforts have been based on the use of agroecology or conventional farming inputs such as organic and inorganic fertilizers, respectively. These have different repercussions on the environment and on crop yields. The use and adoption of conventional farming inputs such as inorganic fertilizers have positive impacts on crop yields, but as well they have also had adverse effects on human health, soils, and water resources. To make things worse, small-scale farmers are often unable to secure several conventional inputs because of the high cost involved (Drechsel et al., 2001; Tittonell et al., 2005; Brock and Foeken, 2006; Schmidhuber and Tubiello, 2007; Lindell et al., 2010a, b; Epule et al., 2012). The debate assumes that organic inputs increase yields and limit environmental degradation while inorganic inputs increase yields but degrade the environment. As such, being that these assertions are not well grounded, it still remains unclear which of agroecology or conventional farming techniques do increase crop yields more.

In the western world and the global south, agricultural production has been based on a dominant agricultural model that is anchored on: (1) mechanization and monoculture, (2) commercial crops, market development and globalization and integration, and (3) inorganic fertilizers and crop hybrid seeds of all types (Matson et al., 1997; Drechsel et al., 2001; Tittonell et al., 2005; Godfray et al., 2010; Epule et al., 2012; Snapp et al., 2010). This model has been associated with problems of agricultural production stagnation and decline in the global south due mainly to problems of poverty and access to these external inputs. Therefore, the potential of crop yield stagnation increases with the poverty level as it is the case in most developing regions of the world (Rosegrant and Svendsen, 1993; Matson et al., 1997; Hossain and Singh, 2000; Adesina et al., 2000; Reid et al., 2003; Lindell et al., 2010a, b; Epule et al., 2012). Alternatively, the agroecology approach may offer a new way forward. It is based on production that uses natural nutrient cycling with little or no synthetic substances and consequently easily accessible to small-scale farmers (Bezner-Kerr et al., 2007; Badgley et al., 2007; Snapp et al., 2010). This approach could lead to crop yields improvements through four pathways: (1) accumulation of organic matter and nutrient cycling through the use of

natural processes; (2) natural control of diseases and pests through the use of nets and predator-prey strategies rather than the use of chemicals; (3) conservation of resources such as water, soil, biodiversity and energy through major processes of sustainable water management through irrigation (Valipour 2014a, b, c, d, e, f, g, 2015; Valipour et al., 2015); and (4) improvement of biological interactions, biodiversity and synergies between agricultural sub-systems. Some of the reasons underlying this new model are based on attempts at answering questions related to ways of feeding the population in the midst of an old model that is not working adequately as reflected in a rise in the prices of inputs such as inorganic fertilizers. In addition to the cost associated with inorganic fertilizers, they also have negative environmental effects.

The present study uses population perceptions to evaluate the relative effects of agroecology and conventional farming techniques on crop yields among small-scale farmers in the Meme and Fako divisions of the South West Region of Cameroon. The analyses of the adoption of agroecology and conventional techniques, the reasons for the adoption of the latter methods, and the coping mechanisms adopted by the farmers are not within the scope of this current study and are simply part of a larger research project on agroecology and conventional farming techniques in parts of Cameroon. This study specifically aimed at verifying the relative effects of agroecology and conventional farming techniques on crop yields in four sites in Cameroon.

## MATERIALS AND METHODS

### Study area

This study was carried out in the Fako and Meme divisions of the South West Region of Cameroon. In each of these divisions, two research sites were selected. The first research site in the Fako division is called the Bonjungo court area. This research site is located at latitude 4.02° N and longitude 9.19° E (Figure 1). The Bonjungo court area has a population of about 11 thousand people. The second site in the Fako division is called Lower Muea. It is located at latitude 4.16° N and longitude 9.23° E (Figure 1). Lower Muea has a population of about 8 thousand people. Both sites in the Fako division have several similarities and characteristics for which they were selected: (1) they are both located on the foot hills of Mount Cameroon where there are fertile volcanic soils (Dounias et al., 2002; Sevink et al., 2004). (2) They both have an equatorial climate that is hot, humid and has persistent rainfall all year round. They have a mean annual temperature of about 18.6° C and an annual rainfall of about 2815 mm. The driest month is December with about 29 mm of rainfall and the wettest month is August with about 488 mm of rainfall. The warmest month is March with temperatures of about 19.7° C while the coldest month is July with temperatures of about 17.3° C. (3) About 80% of their population is comprised of small-scale farmers and their families who depend entirely on agriculture for their livelihoods.

In the Meme division, the first research site was Bole Bakundu. This research site is located at latitude 4.64° N and longitude 9.73° E (Figure 1). Bole Bakundu has a population of about 4 thousand

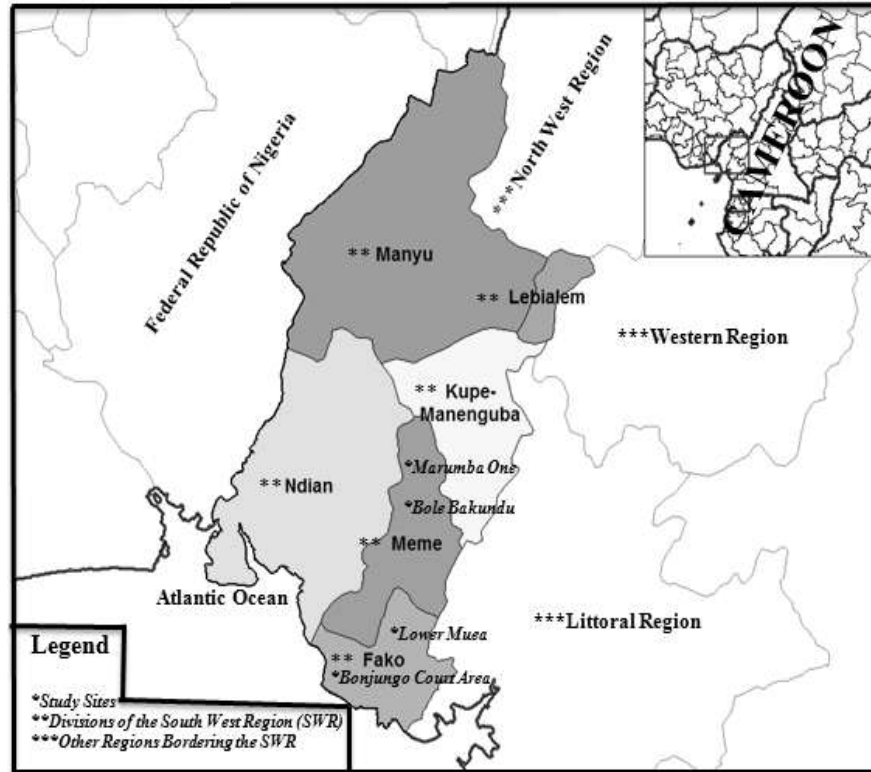


Figure 1. Location of four study sites in the South West Region of Cameroon.

people. The second site in the Meme division is called Marumba one. It is located at latitude 4.63°N and longitude 9.09° E (Figure 1). Marumba one has a population of about 2 thousand people. Both sites in the Meme division have several similarities and characteristics for which they were selected: (1) they are both located on the far eastern slopes of Mount Cameroon where we can find very fertile sandy-loam soils (Dounias et al., 2002; Sevinck et al., 2004); (2) they both also have a tropical climate which is hot, humid and with sufficient rainfall; (3) they both record a mean annual temperature of about 25°C and an annual rainfall of about 864 mm. The driest month is January with about 0 mm of rainfall and the wettest month is August with about 247 mm of rainfall. The warmest month is April with a temperature of about 29.3°C, while the coldest month is December with a temperature of about 22.2°C. About 85% of their population is comprised of small-scale farmers and their families who depend entirely on agriculture for their livelihoods.

#### Data collection

Two focus group discussions (FGDs) were constituted with household heads. One was held in the Bonjungo court area (comprised 8 females and 7 males) and the other was held in Bole Bakundu (comprised 5 females and 10 males). The participants were invited to participate in the discussions through their local chiefs. The discussions were conducted in order to have deeper view points on the relationship between agroecology and conventional techniques of production and crop yields from among small-scale farmers. The discussions were conducted by the researcher and three research assistants. All the objectives of the FGDs were read out and explained to all the participants. In cases where a participant did not understand because of language

barriers, research assistants who spoke Mokpwe and Oroko languages (local languages spoken by the Bakweris and Bakundus of the Fako and Meme divisions, respectively) helped in the translation. Participants in the FGDs had to be fulltime small-scale farmers, they had to be involved in food crop cultivation, they had to be willing to permit the researchers to visit their farms and they were free to opt out at any stage of the discussion. The participants were also asked to give reasons for their opinions during the FGDs. The key themes discussed during the FGDs were: (1) knowledge, use and effects of agroecology techniques on crop yields; (2) knowledge, use and effects of conventional techniques on crop yields.

After the FGDs, a stratified-random one-to-one survey of 200 small-scale farming households was conducted through the administration of 200 questionnaires. Fifty (50) questionnaires were administered in each of the four research sites. However, before the questionnaires were administered, they were pre-tested on three respondents at each research site to ensure that the questions were properly formulated. In cases where questions were ambiguous, these questions were restructured to improve their clarity. The questions concerned the following themes: (1) respondents' age group, gender, level of education, number of years of farming experience, annual income and the number of family members that live and work on the farm; (2) respondents' knowledge and usage of agroecology and conventional farming techniques and how these affect small-scale crop yields. The detailed socio-demographic data collected in this study are shown in Table 1.

#### Data analysis

The FGDs data were analysed using verbatim transcription and

**Table 1.** Socioeconomic and demographic characteristics of respondents.

Variable	Fako division				Meme division				Total	
	Bonjungo court area		Lower Muea		Bole Bakundu		Marumba One		Frequency	%
	Frequency	%	Frequency	%	Frequency	%	Frequency	%		
<b>Gender</b>										
Male	29	58	27	54	24	48	28	56	108	54
Female	21	42	23	46	26	52	22	44	92	46
Total	50	100	50	100	50	100	50	100	200	100
<b>Age groups (Years)</b>										
<25	1	2	0	0	0	0	0	0	1	0.5
25-30	0	0	0	0	0	0	0	0	0	0
31-35	9	18	6	12	8	16	9	18	32	16
36-40	7	14	10	20	7	14	8	16	32	16
41-45	13	26	15	30	17	34	18	36	63	31.5
46-50	12	24	10	20	9	18	8	16	39	19.5
51-60	5	10	8	16	7	14	4	8	24	12
>60	3	6	1	2	2	4	3	6	9	4.5
Total	50	100	50	100	50	100	50	100	200	100
<b>Level of Education</b>										
No Formal	3	6	5	10	7	14	14	28	29	14.5
Primary	31	62	32	64	27	54	22	44	112	56
Secondary	6	12	5	10	8	16	7	14	26	13
>Secondary	10	20	8	16	8	16	7	14	33	16.5
Total	50	100	50	100	50	100	50	100	200	100
<b>Number of years of farming experience (Years)</b>										
5-10	10	20	8	16	8		6	12	32	16
11-15	10	20	9	18	7		8	16	34	17
16-20	0	0	0	0	4		5	10	9	4.5
21-25	20	40	2	4	13		12	24	47	23.5
26-30	10	20	12	24	12		12	24	46	23
>31	0	0	19	38	6		7	14	32	16
Total	50	100	50	100	50		50	100	200	100
<b>Annual income level (CFA)</b>										
<240 K	24	48	20	40	24		22	44	90	45
240k-480 K	7	14	11	22	7		6	12	31	15.5
481K-720 K	3	6	6	12	0		2	4	11	5.5

**Table 1. Cont'd**

721K-960 K	3	6	3	6	3	5	10	14	7
972K-1.2 M	13	26	10	20	11	9	18	43	21.5
> 1.2 M	0	0	0	0	5	6	12	11	5.5
Total	50	100	50	100	50	50	100	200	100
<b>Number of family members that live and work on the farm</b>									
1-2	2	4	5	10	0	15	30	22	11
3-5	33	66	26	52	30	19	38	108	54
6-8	15	30	16	32	14	9	18	54	27
> 8	0	0	3	6	6	7	14	16	8
Total	50	100	50	100	50	50	100	200	100
<b>Agro-techniques</b>									
Agroecology	50	100	42	84	40	42	84	174	87
Conventional	40	80	45	90	47	39	78	171	85.5
Yields 701-1000 kg	20	40	19	38	16	16	32	71	35.5

Wordstat 7 context analysis software. Context analysis enhances the identification of key themes emanating from the discussion. The Wordstat 7 software was used because of its ability to identify themes or relationships in verbatim responses, focus group transcripts or other text sources. It involved four main steps (Adam et al. 2015); 1. identification of the themes, 2. attributing codes to the themes, 3. classification of responses under the themes; and 4. integration of themes and responses into narratives. Also, descriptive and inferential statistical tools were used to analyse the quantitatively derived data using SPSS version 20. Specifically, the descriptive statistical methods included: frequencies, means and percentages based on all the questions under investigation. In terms of inferential statistics, the study used essentially correlation coefficients, coefficients of determination and linear regressions models to investigate the relationship between variables. In computing these equations, the most reported yield level of 701-1000 kg per year was used as the dependent yield variable. This was selected based on the population frequencies as a majority of the respondents reported yields within this range with a total frequency of 71 for both agroecology and conventional farming (Table 1).

The equations used to compute the linear regression models were as follows, considering  $Y_{c701-1000kg}$  as crop yields in the range of 701 to 1000 kg<sup>-1</sup>:

$$Y_{c701-1000kg} = \alpha_0 + \alpha_1 X_{agro\_agr} + \alpha_2 X_{conv\_agr} \dots \dots \dots \text{equation 1}$$

where  $\alpha_1 X_{agro\_agr}$  is the agroecology techniques for different age groups;  $\alpha_2 X_{conv\_agr}$  is the conventional techniques for different age groups.

$$Y_{c701-1000kg} = \alpha_0 + \alpha_1 X_{agro\_edu} + \alpha_2 X_{conv\_edu} \dots \dots \dots \text{equation 2}$$

where  $\alpha_1 X_{agro\_edu}$  is the agroecology techniques for different levels of education;  $\alpha_2 X_{conv\_edu}$  is the conventional techniques for different levels of education.

$$Y_{c701-1000kg} = \alpha_0 + \alpha_1 X_{agro\_fexp} + \alpha_2 X_{conv\_fexp} \dots \dots \dots \text{equation 3}$$

where  $\alpha_1 X_{agro\_fexp}$  is the agroecology techniques for different years of farming experience;  $\alpha_2 X_{conv\_fexp}$  is the conventional techniques for different years of farming experience.

$$Y_{c701-1000kg} = \alpha_0 + \alpha_1 X_{agro\_income} + \alpha_2 X_{conv\_income} \dots \dots \dots \text{equation 4}$$

where  $\alpha_1 X_{agro\_income}$  is the agroecology techniques for different income levels;  $\alpha_2 X_{conv\_income}$  is the conventional techniques for different income levels.

$$Y_{c701-1000kg} = \alpha_0 + \alpha_1 X_{agro\_family} + \alpha_2 X_{conv\_family} \dots \dots \dots \text{equation 5}$$

where  $\alpha_1 X_{agro\_family}$  is the agroecology techniques for different numbers of family members that live and work on the farm;  $\alpha_2 X_{conv\_family}$  is the conventional techniques for different numbers of family members that live and work on the farm.

## RESULTS

### Socio-economic and demographic characteristics of the respondents

Six main characteristics were evaluated (Table 1). Generally, of the 200 small-scale farmers interviewed, 54% (108) were males while 46% (92) were females. According to most of the respondents, when both men and women or couples are involved, the male in the couple is still considered as the main farmer, because men are considered as the main providers of sustenance to their families, while the women either accompany the men to work on the farm or stay at home to take care of children and perform other housewife chores. This distribution heralds the debate that when it comes to issues of ownership and access to land and its resources, men have both ownership and access while women in most cases may be granted access through the men's discretion. Married women do not have unlimited ownership of land and its resources. In terms of the age groups of small-scale farmers, 63 respondents representing 31% of the total belonged to 40 to 45 years age group while the >60 years age group recorded the smallest number of respondents (9 respondents or 4.5%). Results from the FGDs show that the >60 years age group was essentially made of the old who are no longer actively capable of carrying out sustained farming as a result of a reduction in their strength. In relation to level of education, the majority of the respondents representing 56% of interviewees had achieved only primary education. The range of 21 to 25 years of farming experience was the lead category with 47 respondents representing 23.5% of the interviewees. In terms of annual income, the <240 thousand FCFA income group recorded the highest level with 45% or 47 respondents. The generalized observation was that as income levels increased, the number of interviewees decreased. According to the FGDs, the farmers are generally poor and have limited access to expensive farming inputs. Finally, with regards to the family members who live and work on the farm, the results showed that on average, between 3 and 5 persons live and work on the farm. This was reflected in 45% or 108 of all respondents recorded for this category. From the FGDs, this study observed that in all of the study sites, family labour is a key production element as often the farmers depend on the assistance they obtain from their wives and children in order to be able to meet production needs. The ranges presented here include all those living and working on the farm including the farmers, their wives, children, and other dependents.

### Evolution of current and past yields

According to population perceptions, current arable crop production yields (December, 2014) in the four study

sites are declining. This is evident as of the 200 respondents, 178 respondents said that their current yields were declining, while 8 said they were stagnant, 14 said they were increasing, 0 said they are unpredictable, 0 said they have no idea and 0 said others. In terms of yields during the past decade, 154 respondents said they were increasing while 46 said they were declining. The majority of the respondents representing 71 persons had crop yields between 701 and 1000 kg year<sup>-1</sup> (Figure 2a, b, and c). These results presented here underscore the fact that current small-scale farming crop yields are lower than they were 10 years ago. These results are consistent with the FGDs.

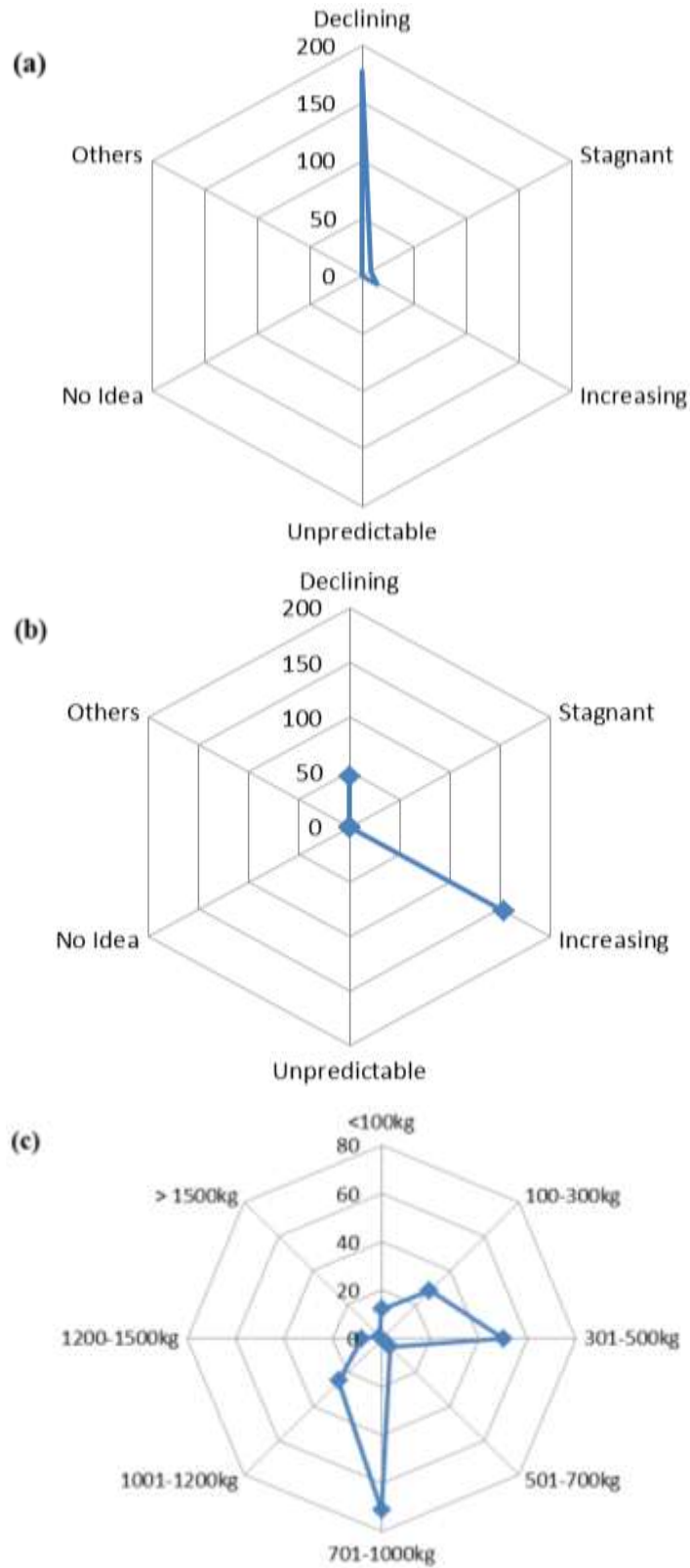
### Agroecology and conventional yields, scatter plots, correlation coefficients and coefficients of determination

It was observed that the 41 to 45 years age group had the highest number of 25 respondents representing 35.21% of respondents having their yields comprised between 701 and 1000 kg year<sup>-1</sup> (Figure 3a).

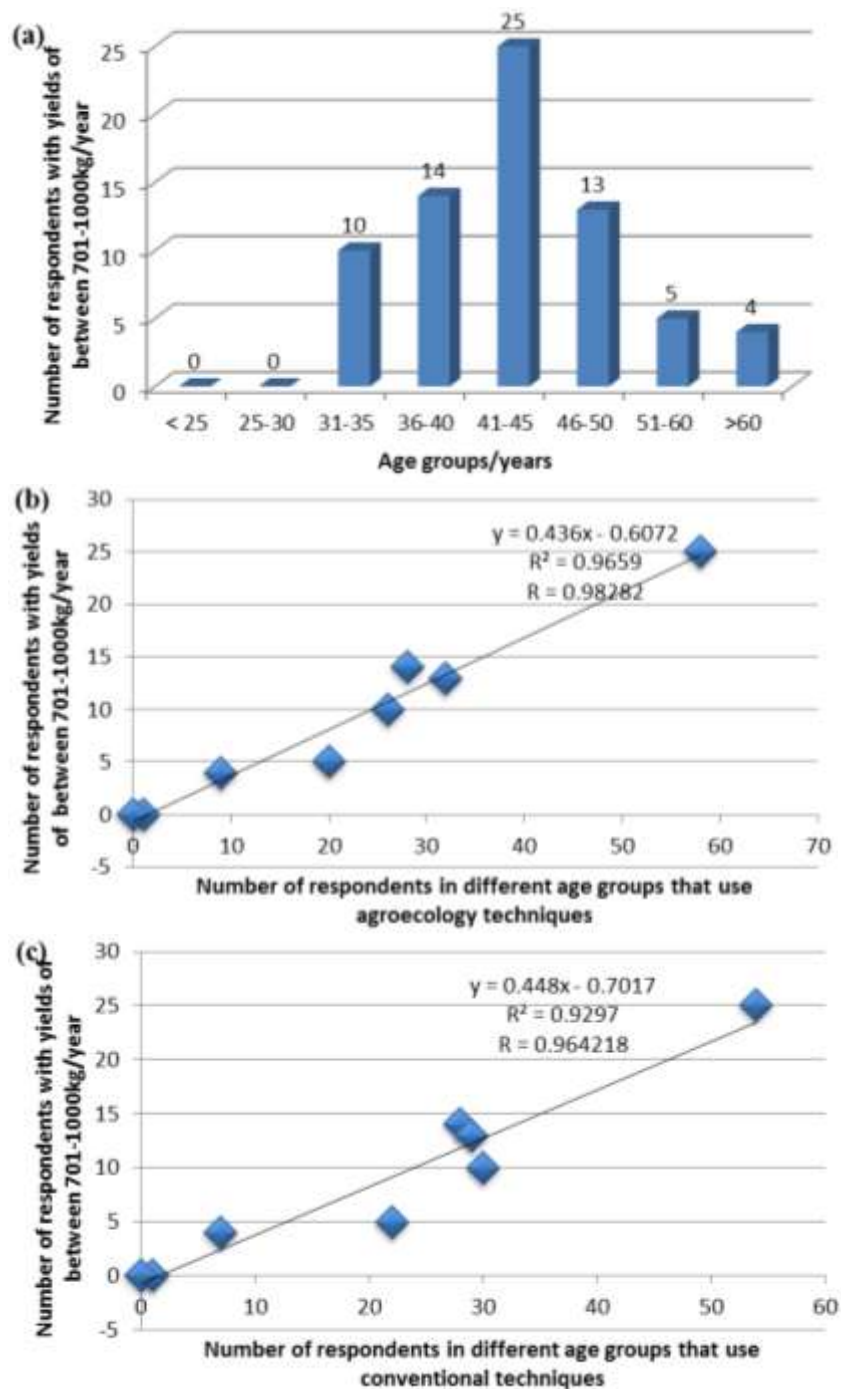
As presented earlier, 71 respondents have yields of between 701 and 1000 kg per year. When it comes to how this trends for different age groups, it can be observed that the 41 to 45 years age group has the highest number of respondents of 25 (Figure 3a). The latter age group represents the active age group and this may be the reason why this age group dominates the maximum yield range. Results from the FGDs also go a long way to confirm this assertion.

In terms of the contributions of agroecology and conventional farming to crop yields in the four study sites, the following observations were made. A scatter plot of the number of respondents in different age groups that use agroecology techniques against the number of respondents with yields comprised between 701 and 1000 kg per year showed a perfect positive relationship.

This was expressed by a correlation coefficient (R) of 0.98 and a coefficient of determination (R<sup>2</sup>) of 0.96 (Figure 3b). These values are higher than 0.96 and 0.92 obtained from the scatter plot of the number of respondents in different age groups that use conventional techniques against the number of respondents with yields of between 701 and 1000 kg per year (Figure 3c and Table 2). Consequently, these results showed that the yields from the four study sites were higher under agroecological than conventional techniques for different age groups. The higher correlation coefficient depicted a more positive relationship between the number of respondents that use agroecological techniques for different age groups and yields while the higher coefficient of determination for agroecology techniques also means that about 96% of the changes in yields can be explained by the linear relationship between yields and agroecology techniques. For conventional farming techniques, the coefficient of determination was about



**Figure 2.** (a) Respondents perception of the status and evolution of current crop yields. (b) Status and evolution of past crop yields in the last decade. (c) Actual current mean annual crop yields in kilograms based on population perceptions of 200 respondents in all four study sites.



**Figure 3.** (a) Evolution of both agroecology and conventional yields for different age groups. (b) Scatter plots of maximum yields and age groups of farmers under agroecology techniques. (c) Scatter plots of maximum yields and age groups of farmers under conventional techniques.

92% and inferior to 0.96 obtained for agroecological techniques. Therefore, statistically, it is concluded that agroecology techniques are more responsible for yield increases than conventional farming techniques for the variables presented.

In the area of level of education, this study also found that of the 71 respondents with yields that comprised between 701 and 1000 kg, the majority of 31 have only attained primary education (Figure 4a). This goes to support the view from the FGDs that a majority of the

**Table 2.** Correlation coefficients (R) and coefficients of determination ( $R^2$ ) between yield range with highest number of respondents and selected variables.

Yield range	Socio-demographic variables	R	$R^2$	Remarks
701-1000 kg year <sup>-1</sup>	Age group under agroecology	0.98	0.96**	Positive and dominant
	Age group under conventional	0.96	0.92*	Positive and less dominant than above
	Level of education under agroecology	0.99	0.97**	Positive and dominant
	Level of education under conventional	0.98	0.96*	Positive and less dominant than above
	Years of farming experience under agroecology	0.88	0.77**	Positive and dominant
	Years of farming experience under conventional	0.84	0.72*	Positive and less dominant
	Income level under agroecology	0.99	0.97*	Positive and less dominant than below
	Income level under conventional	0.99	0.98**	Positive and dominant
	Family members that live and work on the farm under agroecology	0.98	0.96*	Positive and less dominant than below
	Family members that live and work on the farm under conventional	0.98	0.97**	Positive and dominant

\*\*Positive and dominant relationship, \*Positive but less dominant relationship.

respondents in the study sites have not studied beyond the primary level of education.

The scatter plots representing the relationship between the number in different education levels involved in agroecological techniques and the number with the yield range of 701 and 1000 kg year<sup>-1</sup> (Figure 4b) are more responsible for increases in crop yields than the scatter plots between the number in different levels of education that use conventional techniques and the number with yields between 701 and 1000 kg year<sup>-1</sup> (Figure 4c and Table 2). This is observed as the agroecology yield scatter plot for different levels of education has a correlation coefficient of 0.98 and a coefficient of determination of 0.97 (Figure 4b). These were higher than the correlation coefficient and coefficient of determination for the relationship between yields and conventional farming that were 0.98 and 0.96, respectively (Figure 3c and Table 2). Again, it is observed that, for different levels of education, agroecology techniques seem to be more associated to yield increases.

Out of the 71 respondents that have yields of between 701 and 1000 kg year<sup>-1</sup>, the highest

category of 23 respondents had between 21 and 25 years of farming experience (Figure 5a). Here also, agroecological techniques were seen to affect yields in a positive direction for different numbers of years of farming experience than for conventional techniques of farming (Figure 5b and c and Table 2).

In terms of income levels, the respondents, in their majority, were generally poor with most of them belonging to the annual income level category of <240 thousand FCFA year<sup>-1</sup> (Figure 6a). Unlike with the other socioeconomic variables, the scatter plot of number in different income groups that use agroecological techniques and the number of respondents with yields between 701 and 1000 kg year<sup>-1</sup> had a correlation coefficient of 0.99 and an inferior coefficient of determination of 0.97 (Figure 6b) when compared with a correlation coefficient of 0.99 and a coefficient of determination of 0.98 for the relationship between number in different income groups that use conventional techniques and number with yields of between 701 and 1000 kg year<sup>-1</sup> (Figure 6c and Table 2). The implication here is that, when it concerns different income

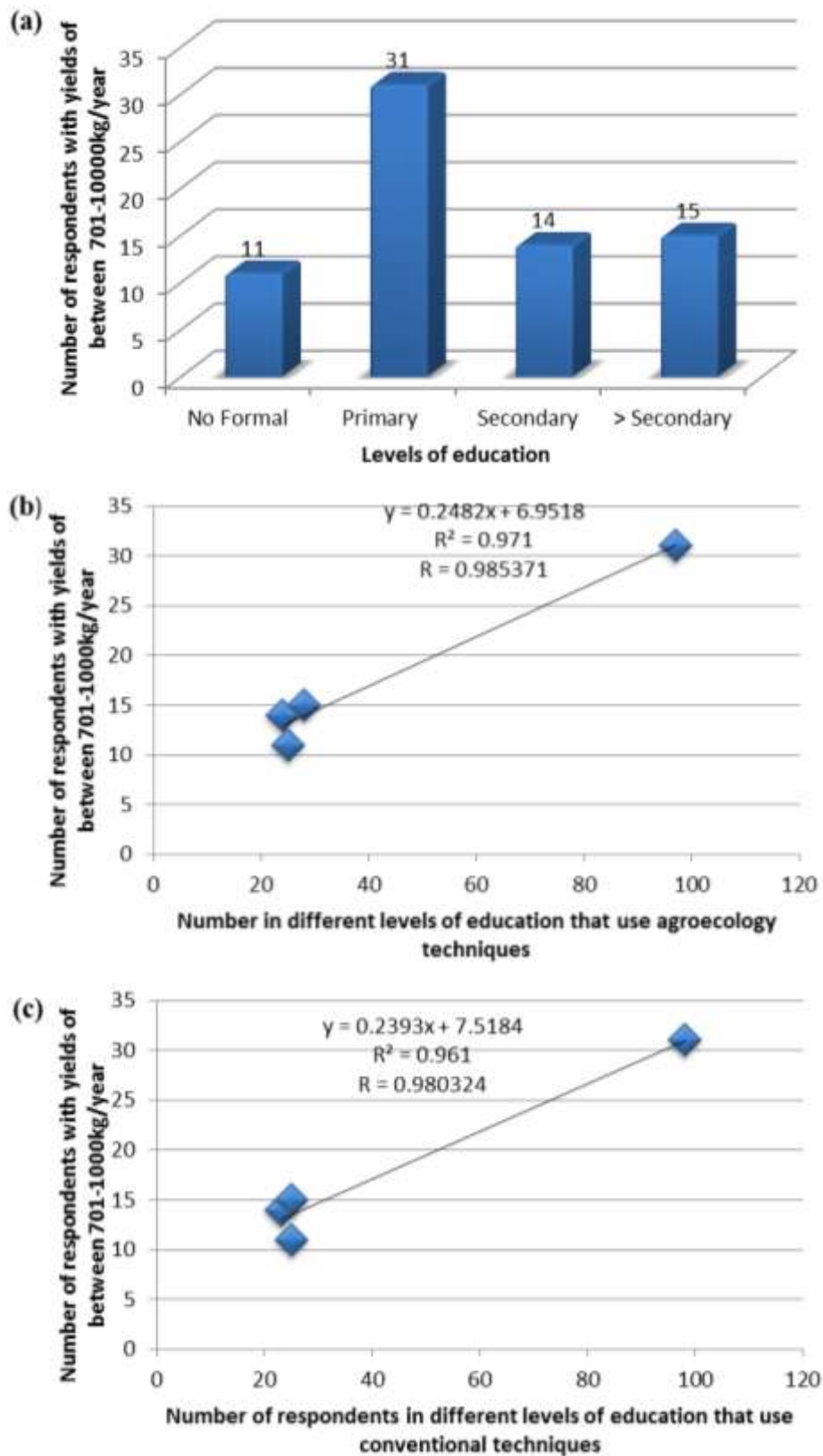
groups, conventional farming systems seem to increase yields slightly more than agroecology systems.

Concerning the number of family members that live and work on the farm, this study has found that, for all four study sites and with respect to the 71 respondents that had yields of between 701 and 1000 kg per year (Figure 7a), a majority of 35 belong to the group of 3 to 5 persons that live and work on the farm. In terms of the relative contributions of agroecology and conventional farming to yields, the yields under conventional farming techniques are more important than those under agroecology techniques. The conventional farming scatter plot has a coefficient of determination of 0.98 (Figure 7b and c and Table 2) that was statistically higher than that of agroecology farming.

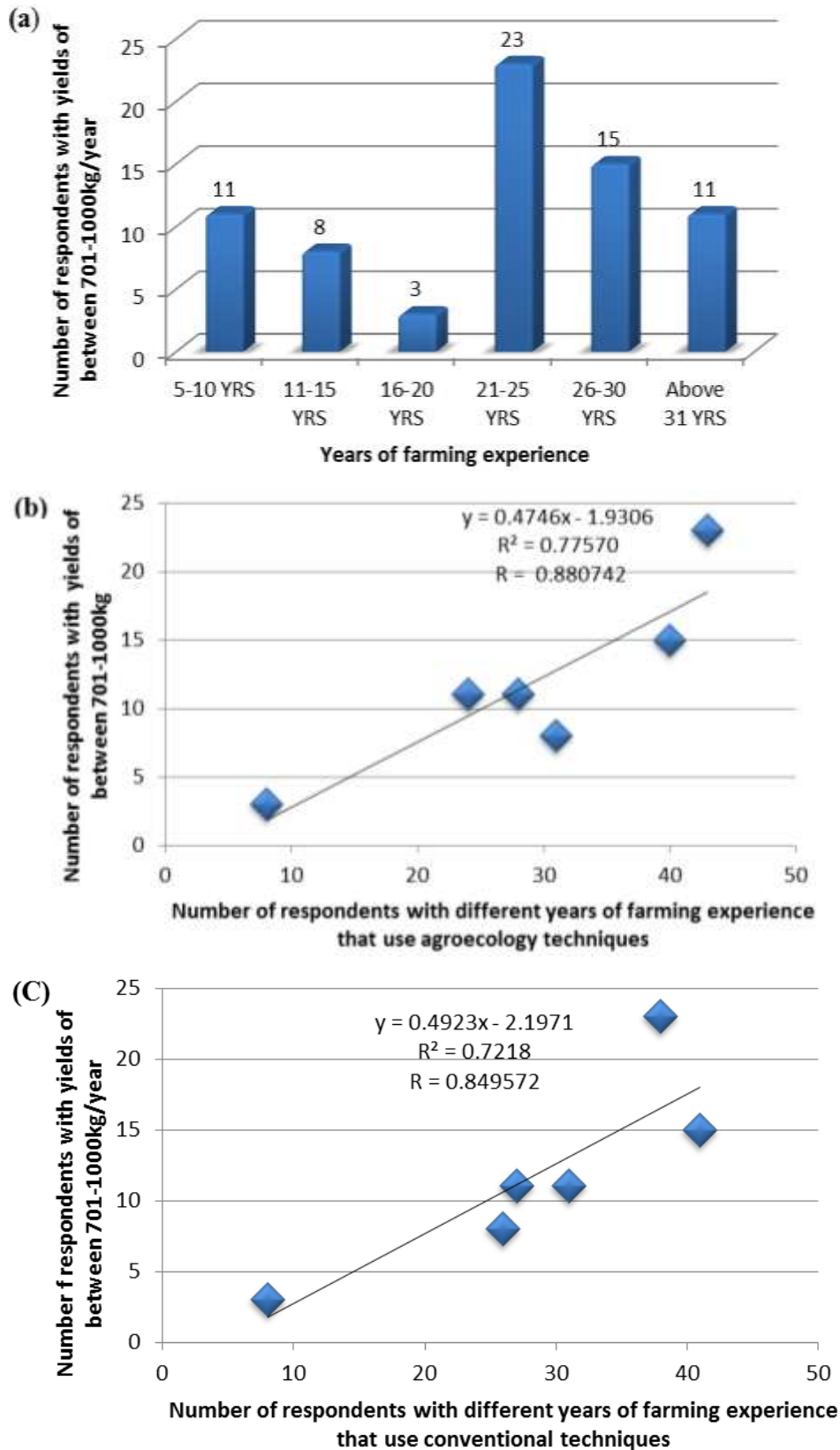
### Multiple linear regression models

Different linear regression models computed based on the different socio-economic variables under study tended to be very consistent with the

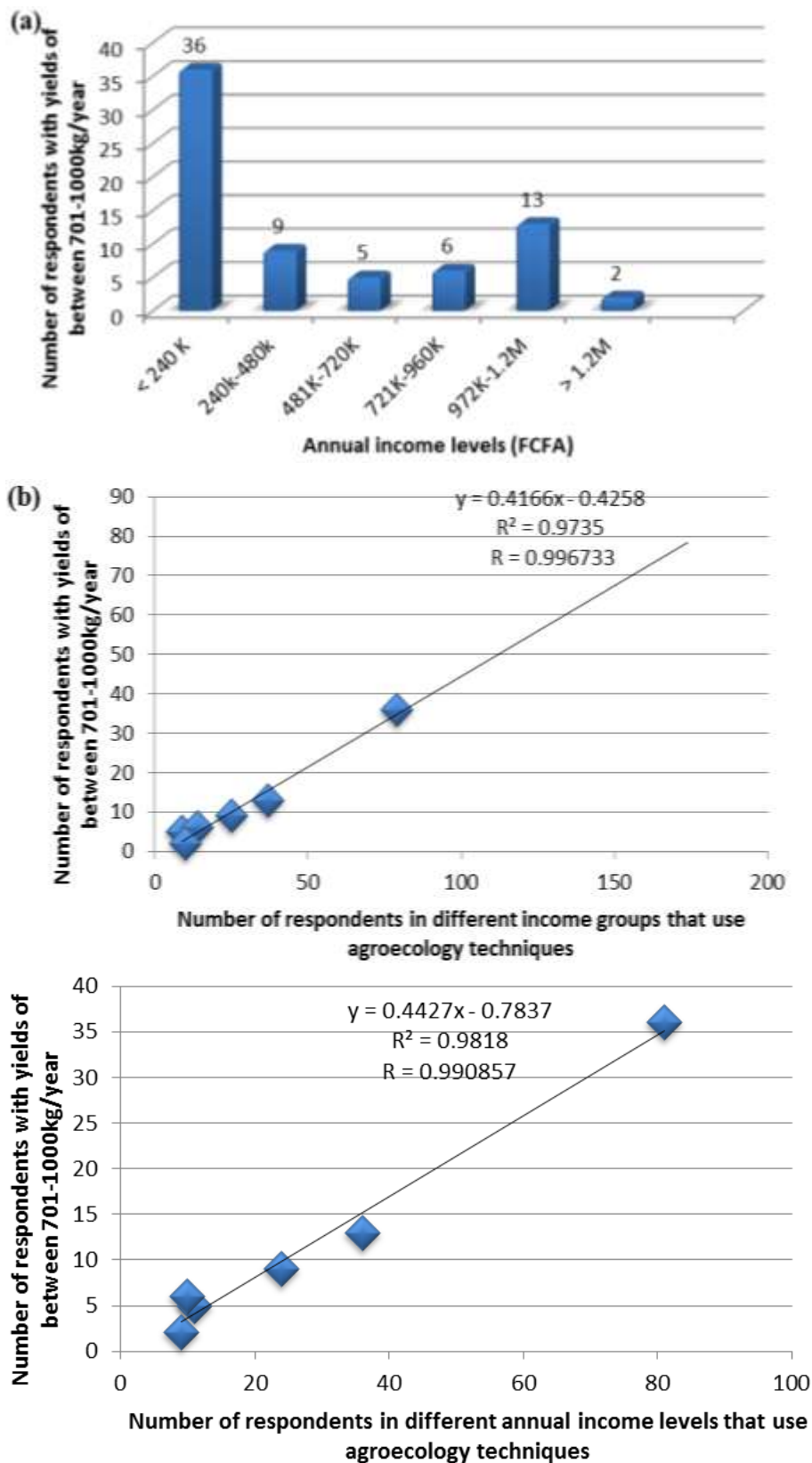




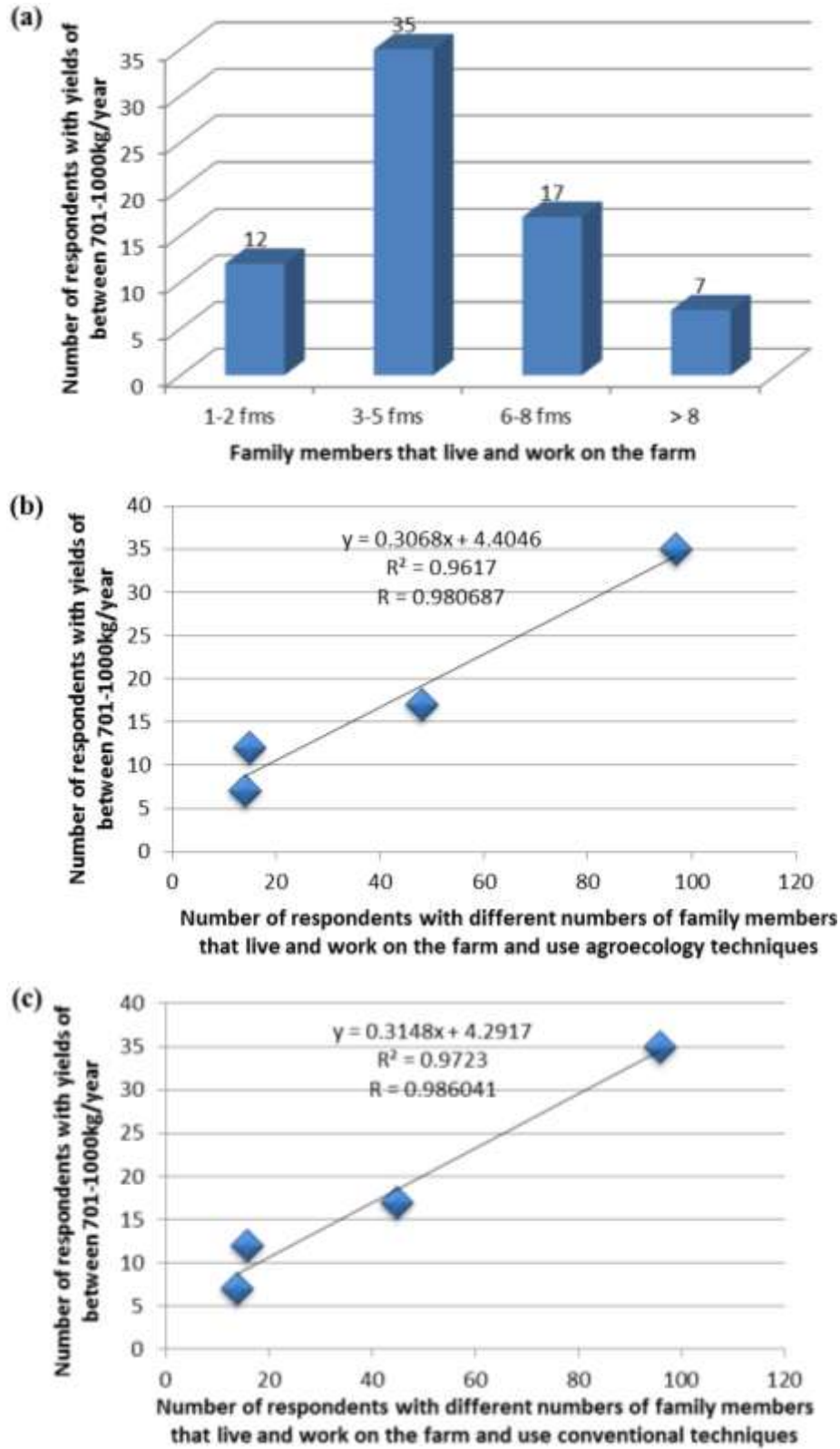
**Figure 4.** (a) Evolution of both agroecology and conventional yields for different levels of education. (b) Scatter plots of maximum yields and level of education of farmers under agroecology techniques. (c) Scatter plots of maximum yields and level of education of farmers under conventional techniques.



**Figure 5.** (a) Evolution of both agroecology and conventional yields for different number of years of farming experience. (b) Scatter plots of maximum yields and years of farming experience of farmers under agroecology techniques. (c) Scatter plots of maximum yields and years of farming experience of farmers under conventional techniques.



**Figure 6.** (a) Evolution of both agroecology and conventional yields for different annual levels of income. (b) Scatter plots of maximum yields and annual income of farmers under agroecology techniques. (c) Scatter plots of maximum yields and annual income of farmers under conventional techniques.



**Figure 7.** (a) Evolution of both agroecology and conventional yields for numbers of family members the live and work on the farm. (b) Scatter plots of maximum yields and numbers of family members that live and work on the farm for farmers under agroecology techniques. (c) Scatter plots of maximum yields and numbers of family members that live and work on the farm for farmers under conventional techniques.

**Table 3.** Regression model of the predictors of mean annual yields based on different age groups.

Independent variable	Number of respondents that use agroecology techniques	Number of respondents that use conventional techniques
Coefficient	0.69	-0.27
Standard error	0.25	0.26
t-value	2.71**	-1.0*
p-value	0.04**	0.36*
Rank of t-value	1	2
Rank of p-value	1	2

R = 0.97; R<sup>2</sup> = 0.97; Adjusted R<sup>2</sup> = 0.96; f statistics = 85.63.

**Table 4.** Regression model of the predictors of mean annual yields based on different levels of education.

Independent variable	Number of respondents that use agroecology techniques	Number of respondents that use conventional techniques
Coefficient	1.15	-0.87
Standard error	0.81	0.79
t-value	1.40**	-1.10*
p-value	0.39**	0.47*
Rank of t-value	1	2
Rank of p-value	1	2

R = 0.99; R<sup>2</sup> = 0.98; Adjusted R<sup>2</sup> = 0.96; f statistics = 37.82.

**Table 5.** Regression model of the predictors of mean annual yields based on number of years of farming experience.

Independent variable	Number of respondents that use agroecology techniques	Number of respondents that use conventional techniques
Coefficient	0.43	0.04
Standard error	0.50	0.54
t-value	0.86**	0.09*
p-value	0.46**	0.93*
Rank of t-value	1	2
Rank of p-value	1	2

R = 0.88; R<sup>2</sup> = 0.77; Adjusted R<sup>2</sup> = 0.62; f statistics = 5.20.

results from the scatter plots, the correlation coefficients and the coefficients of determination. In all of these models, yields constitute the dependent variable and the number of respondents that use agroecology and conventional techniques constitute the independent variables.

For example, the regression model that explains the predictors of yield based on different income groups showed that the number of respondents that use agroecology techniques had a t-value of 2.71 and a p-value of 0.04. These values were different from the t-value of 1.0 and p-value of 0.36 for the number of respondents that use conventional techniques. The higher t-value of 2.71 for agroecology shows that

agroecology contributes more to yields than conventional farming. The parallel p-value of 0.04 is the lower of the two and depicts the fact that the likelihood of having outcomes that are different from the current is highly limited and thus, these results are reliable (Table 3). For the effects of agroecology and conventional techniques on yields based on: levels of education (Table 4), number of years of farming experience (Table 5) the results were consistent as agroecology techniques were seen to influence yields more due to larger t-values and smaller p-values.

However, in the case of the effects of agroecological and conventional techniques on yields based on income levels and the number of family members that live and

**Table 6.** Regression model of the predictors of mean annual yields based on different income levels.

Independent variable	Number of respondents that use agroecology techniques	Number of respondents that use conventional techniques
Coefficient	0.08	0.36
Standard error	0.49	0.47
t-value	0.17*	0.76**
p-value	0.88**	0.50**
Rank of t-value	2	1
Rank of p-value	2	1

R = 0.99; R<sup>2</sup> = 0.98; Adjusted R<sup>2</sup> = 0.96; f statistics = 81.66.

**Table 7.** Regression model of the predictors of mean annual yields based on the number of family members that live and work on the farm.

Independent variable	Number of respondents that use agroecology techniques	Number of respondents that use conventional techniques
Coefficient	-0.93	1.27
Standard error	0.94	0.96
t-value	-0.98*	1.31**
p-value	0.50*	0.41**
Rank of t-value	2	1
Rank of p-value	2	1

R = 0.99; R<sup>2</sup> = 0.98; Adjusted R<sup>2</sup> = 0.95; f statistics = 35.14.

work on the farm, this study observed that conventional farming techniques were dominant. For example, in the case of different income groups, conventional farming techniques had a higher t-value of 0.76 and a lower p-value of 0.50 (Table 6) meaning that conventional farming techniques affect yields more than agroecological techniques. This again can be explained by the fact that conventional techniques or inputs were not free and need to be purchased and that was why, when income increases among respondents, the rates of usage tended to be high. Also, for the final model based on the number of family members that live and work on the farm, this study also found out that, conventional farming techniques tended to affect yields more than agroecology as seen in a higher p-value of 1.31 and a lower t-value of 0.41 for conventional farming when compared with agroecological practices (Table 7).

## DISCUSSION

With the exception of socio-demographic variables like income levels and the number of family members that live and work on the farm, for all the other social demographic variables and characteristics, crop yields tended to increase more positively under systems of agroecology than conventional farming. Income levels tended to increase yields more under conventional farming

techniques because most conventional inputs are not freely available and farmers who are richer are often able to purchase inputs such as inorganic fertilizers, hydrides, machines, etc. Agroecology farming systems on the other hand were not highly income driven as is the case with conventional inputs because they are often freely available from the environment (Nkamleu and Adesina, 2000; Palm et al., 2001; Negi, 2014). In general, agroecology seems to be generally more favorable but again, both techniques, that is, agroecology and conventional techniques are very highly rated with correlation coefficients and coefficients of determination generally above 70%. This result is consistent with recent findings from across Africa that showed that both agroecology and conventional farming techniques are generally being used in Africa. However, the problem with agroecology is that it is still unable to support high yields and this is linked to the fact that this system of production has not been properly valorised in most African countries and many farmers still simply farm under natural conditions without taking into account agroecological practices.

These findings are consistent with several other studies on the effects of agroecology and conventional farming on yields. It has been suggested that agroecology techniques alone are unable to trigger sufficient yields if they are not associated with conventional techniques (Chivenge et al., 2009). Using organic fertilizers as an

example of agroecology techniques, it has been further substantiated that the current levels of organic fertilizers usage in most of Africa cannot support an increase in production beyond levels at which galloping populations can be fed (Borlaug, 2000; Tilman et al., 2002; Ayuke et al., 2011). Epule et al. (2015) argued that organic fertilizers can increase production to a certain threshold beyond which further increase can only be achieved through the wise application of inorganic fertilizers. The best scenario for maximum yields is when organic inputs such as manure are combined with inorganic inputs such as nitrogen, potassium and calcium fertilizers (NPK) (Pichot et al., 1981; Bationo and Mokwunye, 1991; Bado et al., 1997; Adesina et al., 2000; Bado et al., 2007; Folefack, 2009). To further support this, in the North West of Ethiopia, Demelash et al. (2014) present findings from an entirely experimental approach in which a combination of 6 t compost ha<sup>-1</sup> year<sup>-1</sup> and 34.5 to 10 kg N-P ha/year produced the highest crop yields of about 521% when compared with other scenarios that had either only organic or inorganic inputs.

The observation that agroecology techniques were dominantly used by the respondents interviewed in this study was also consistent with other previous studies. For example, organic fertilizers are freely available and can be easily obtained from the environment. In poor communities in Africa in general and Cameroon, particularly, where access to conventional inputs is often limited by purchasing power, organic resources remain the critical nutrient sources for most small scale-farmers who are generally unable to secure large quantities of inorganic inputs (Rosegrant and Svendsen, 1993; Matson et al., 1997; Hossain and Singh, 2000; Reid et al., 2003; Lindell et al., 2010a, b; Palm et al., 2010; Godfray et al., 2010; Epule et al., 2012).

One major problem of food crop production in Cameroon is related to inadequate access to fertilizers and high yielding varieties and poor water and soil management (Henao and Baanante, 2006). This observation explains why agroecology techniques that promote natural systems interactions and inputs are more dominant in the study sites than conventional techniques. The problem is that, agroecology systems in Cameroon have not been properly valorised to levels where small-scale farmers can obtain maximum benefits from their use. Most of the systems described as agroecology in this study are actually natural as in most cases the farmers simply clear up the land and then plant the crops without any additional inputs. In most cases, the use of agroecology techniques is accidental as most farmers tend to use the latter because of lack of access to conventional techniques. The advantages of agroecology techniques are not being fully experienced by farmers because the scale of utilization and level of valorization are low. The farmers need to be educated on the various ways of valorising agroecology inputs such as: the establishment of pilot agroecology farms; usage of various natural

substances such as grass and food wastes to produce compost and organic manure; water and soil management; the use of prey-predator relationships and intensified usage of animal droppings and urine to control pests instead of using lethal pesticides (Epule et al., 2015).

Agroecology inputs such as compost and manure are capable of supplying the much needed crop nutrients and are more accessible because they are free and they are not sources of stream and river pollution as it is observed with inorganic or mineral fertilizers (Lindell et al., 2010a, b; Dubois, 2011; Krawinkel, 2012). As such, well valorised organic farming systems will go a long way to enhance soil fertility, biodiversity, soil organic carbon and nitrogen (Liu et al., 2009).

In the midst of the advantages of organic fertilizers, many studies have argued that in terms of the net effects of optimally managed organic or agroecology farms on yields, organic farms produce lower yields per unit area when compared with inorganic or conventional farms (Lal, 2006). These findings were later confirmed by Seufert et al. (2012) after Badgley et al. (2007) argued that organic or agroecology farming systems are more productive than conventional farms, "...organic agriculture has the potential to contribute quite substantially to the global food supply, while reducing the detrimental environmental impacts of conventional agriculture". In fact, Seufert et al. (2012) criticised Badgley et al. (2007) findings on the following grounds: (1) It included organic crop yields from farms experiencing large inputs of nitrogen from manure; (2) They used less representative low conventional yields; (3) They failed to consider yield reductions over time due to rotations with non-food crops; (4) Double counting of high organic yields; and (5) Extensive use of unverifiable data from grey literature sources and equal weighting of the latter with more rigorous studies.

A major dilemma in the current status of agroecology is whether it will solve the twin problems of providing more food to mankind and at the same time minimising environmental foot prints. This is because the Badgley et al. (2007) study that asserts increase in food production under agroecological conditions has been criticised on grounds of double counting and for not adequately differentiating between agroecological and conventional farms. The Seufert et al. (2012) also fails to deliver by not including any comparisons between conventional and organic yields in Africa as the meta analysis focuses on North America, Central America, Western Europe, and parts of South East Asia. It is more appropriate to say there are inadequate studies on this topic in Africa. Generally, the Seufert et al. (2012) paper takes on a more pessimistic perspective on the prospects of organic systems. However, recent studies based on a synthesis of the existing literature on the use of agroecology and conventional farming methods in Africa by Kearney et al. (2012) and Epule et al. (2015) argue that organic farming

can only support yields to a certain threshold beyond which further increase in yields can only be attained through the application of inorganic fertilizers.

## Conclusion

This study has found that agroecology techniques of farming seem to contribute more to crop yields among small scale peasant farmers in the four study sites. However, exceptions exist when scenarios of the effects of income and the number of family members that live and work on the farm are concerned. Here, conventional farming techniques seem to contribute more to yields. Generally, the performance of both techniques is higher than 70% with agroecology related techniques being slightly dominant. The key problem facing agroecology related techniques now is the low level of valorisation. Observations and FGDs show that, what is currently termed agroecology in most of the study sites is simply production under natural condition without a clear valorisation of composts, organic manure, prey-predator relationships inter alia. This study defines valorisation of agroecology as the complete package of techniques related to not only adding value to different agroecology options like manure, compost, animal droppings and plant residues but also training the farmers on the different ways by which they can obtain and increase their use of these elements at little or no costs. Therefore, through training and capacity building of the small scale peasant farmers on the various ways of obtaining and using agroecology inputs, valorisation can be achieved. In the main time, both conventional and agroecology techniques should be used in the study sites to assure maximum yields.

For issues such as the usage levels of agroecology and conventional farming methods, the reasons for the adoption of the different methods of agroecology and conventional methods and various coping mechanisms are all worth verifying further. Farmers using mainly conventional techniques could be persuaded to integrate progressively certain agroecological techniques primarily because this has the ability of lowering the negative externalities (e.g. on the environment including water resources) associated with dominantly conventional techniques. One major way of encouraging this might be to build on people's community solidarity especially if more farmers can be convinced that doing this would have positive effects on their own families and neighbours' families.

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## Conflict of interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

## Common bean evapotranspiration estimated by orbital images

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Evapotranspiration quantification is essential for water management in irrigated crops because it represents the crop water demand, and knowledge prevents water and energy waste, besides productivity breaks. This study aimed to estimate common bean actual evapotranspiration through orbital images, using the simple algorithm for evapotranspiration retrieving (SAFER) algorithm. In order to achieve this, actual evapotranspiration (ET) and crop coefficient (Kc) were estimated in a bean crop (BRS style) irrigated by center pivot at Distrito Federal (PAD/DF), Brazil. ETa was determined by SAFER energy balance model, using images from Landsat 8 (OLI/TIRS) and climatic variables. Evapotranspiration reference was also determined by Penman-Monteith method (FAO56). Assessments were conducted on five periods during crop development stages, coinciding with the passage of the satellite in orbit 221/71 (06/30; 07/16; 08/01; 08/17 and 09/02/2014). ET values equal to 1.69, 2.52, 3.21, 3.58 and 2.63 mm day<sup>-1</sup> were found for 06/30, 07/16, 08/01, 08/17 and 09/02/2014 periods. ETa values obtained by the SAFER model were on average 4.19% lower than the estimated values from FAO-56 default method. Satellite images and SAFER algorithm use is recommended to estimate bean crops ET.

**Key words:** Energy balance, *Phaseolus vulgaris* L., SAFER, hydro demand.

### INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an agricultural product appreciated by Brazilians. It is considered staple food and one of the main protein sources in the population diet. Although this culture is characterized as one of the main crops in Brazil (Cunha et al., 2013), it is often grown as second harvest, which,

in much of the country, is when the drought begins. Thus, irrigation use is essential for bean growth. Plant evapotranspiration rates knowledge is of fundamental importance for the rational management of water resources, contributing to the increasing agricultural production in irrigated areas. Evapotranspiration (ET) can

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be defined as the total water loss in the soil-plant system caused by the atmosphere, from the combination of evaporation and transpiration processes (Lopes et al., 2014).

There are different methods to estimate evapotranspiration (ET). Remote sensing techniques have been applied effectively (Teixeira, 2012b; Teixeira et al., 2012c), as they allow estimates over large areas without the need of quantifying other hydrological processes (Andrade et al., 2010). ET estimates can be obtained from remote sensing images, applying algorithms combined with physical models (Andrade et al., 2014; Lopes et al., 2014), highlighting surface energy balance algorithm for land (SEBAL) (Bastiaanssen et al., 1998), mapping evapotranspiration at high resolution with internalized calibration (METRIC) (Allen et al., 1998) and simple algorithm for evapotranspiration retrieving (SAFER) (Teixeira et al., 2013a).

SAFER is an algorithm whose non mandatory advantage is to use the thermal band, having the possibility of applying meteorological data from different station types (meteorological, conventional and automatic). Features such as these are important to enable energy balance, evapotranspiration and water productivity components historical trends assessment on a large scale over the years, given that automatic sensors are relatively recent advances in instrumental technology (Teixeira et al., 2013b). SAFER is highlighted as a simplified algorithm (Teixeira, 2012a). Thus, this study aimed to estimate bean actual evapotranspiration through orbital images, using the SAFER algorithm.

## MATERIALS AND METHODS

The study was conducted in an 41 ha area irrigated by center pivot, in the period from 30 May, 2014 to 10 September of 2014, in a commercial farm at the Federal District Directed Settlement Program (PAD/DF, Brazil) (15° 56' 38.97" S; 47° 28' 16.25" W; 880 m). According to Koppen (Codeplan, 1984), climate is classified as Aw tropical, with rainfall concentrated in summer (October to April) and dry season in winter (May to September). The analyzed cultivar was of common beans (BRS Style), consisting of erect architecture and high yield potential. Sowing was mechanically held on 05.30.2014, with spacing of 0.50 m between rows and 11.4 seeds per meter. In order to estimate the actual evapotranspiration (ET), Landsat 8 satellite images (OLI/TIRS, orbit/point 222/71) were used, which were acquired from free stock images of the United States Geological Survey - USGS (2014). The OLI product consists of nine multispectral bands with spatial resolution of 30 m, in which data are collected with 30 m per pixel. TIRS instrument thermal bands show more accurate surface temperatures, and data are collected with 100 m per pixel. The images selected for the study were taken at 30 days after sowing (DAS) on 06.30.2014, at 46 DAS on 07.16.2014, at 78 DAS on 08.17.2014 and at 94 DAS on 02.09.2014.

Evapotranspiration reference (ET<sub>0</sub>) was determined by the method of Penman Monteith, using climate data from weather stations provided by Irriger (Irrigation Management Engineering), which is located 20 km from the area. Raster function calculator

was used as programming and calculation tool, being available on the System Manager software Geographic Information (GIS), which allows modeling calculation and application using raster data. In SAFER, albedo surface (α<sub>s</sub>) was estimated according to the albedo of the atmosphere on the top (α<sub>0</sub>) (Equation [1]):

$$\alpha_s = a \cdot \alpha_0 + b \quad [1]$$

Where a and b are regression coefficients corresponding to the values of 0.7 and 0.006, respectively (Teixeira, 2010). Surface temperature (T<sub>0</sub>) was estimated by Equation 2, which is described by the equation:

$$T_o = a * \text{mean TKelvin} + b \quad [2]$$

Where a and b are correction coefficients, whose values are 1.11 and -31.89, respectively (Teixeira, 2010). Afterwards, normalized difference vegetation index (NDVI) was estimated by (Equation [3]):

$$\text{NDVI} = \frac{\text{IVP}-V}{\text{IVP}+V} \quad [3]$$

Where IVP is infrared light reflectance near V and reflectance in the red band. Subsequently, albedo surface data (α<sub>0</sub>), surface temperature (T<sub>0</sub>) and Normalized Difference Vegetation Index (NDVI) were used to calculate ET/ET<sub>0</sub> ratio instantaneous values by (Equation [4]):

$$\frac{ET}{ET_0} = \exp \left[ a + b \left( \frac{T_0}{\alpha_0 + \text{NDVI}} \right) \right] \quad [4]$$

The "a" coefficient was used with the value of 1.0 (Hernandez et al., 2012; Teixeira et al., 2013a), which was determined for São Paulo state Northwestern region (Noroeste Paulista), proving to be well adapted to the conditions of the studied area. The "b" coefficient was obtained by Teixeira (2010) and corresponds to the value of -0.008. ET<sub>0</sub> is the reference evapotranspiration, given in millimeters per day (mm d<sup>-1</sup>) by Penman-Monteith method, according to FAO Bulletin N°. 56 (Allen et al., 1998).

$$ET_0 = \frac{0.408 X (Rn-G) + \left[ \gamma x \left( \frac{900}{T+273} \right) x u_2 x (e_s - e_a) \right]}{\Delta + \gamma x (1+0.34 x u_2)} \quad [5]$$

Where Rn is the crop surface radiation balance (MJ m<sup>-2</sup> day<sup>-1</sup>), G is the heat flow density in the soil (MJ m<sup>-2</sup> day<sup>-1</sup>), T is air average daily temperature (°C), u<sub>2</sub> is the wind speed at 2 m (ms<sup>-1</sup>), e<sub>s</sub> is the air saturation vapor pressure (kPa) and air actual vapor pressure (kPa), e<sub>s</sub> - e<sub>a</sub> is the air saturation vapor pressure deficit (kPa), Δ is the gradient of the air vapor pressure curve in the atmosphere (kPa °C<sup>-1</sup>) and γ is the psychrometric constant (kPa °C<sup>-1</sup>). Subsequently, ET/ET<sub>0</sub> ratio instantaneous values were multiplied by ET<sub>0</sub> daily values, obtaining the actual evapotranspiration (ET):

$$ET_a = \frac{ET}{ET_0} \cdot ET_0 \quad [6]$$

Bean crop potential evapotranspiration estimation was obtained according to the method in FAO Bulletin N°. 56 (Allen et al., 1998), by Equation 7:

$$ET_c = ET_0 \cdot Kc \quad [7]$$

Where ET<sub>c</sub> is the crop evapotranspiration (mm/day); ET<sub>0</sub> is the reference evapotranspiration (mm/day) and K<sub>c</sub> is the crop coefficient (dimensionless) - FAO N°. 56 (Allen et al., 1998).

Method performance when estimating evapotranspiration by the algorithm (SAFER) was evaluated by correlating the measured data

**Table 1.** Period of days for each bean crop development stage.

Stage/Period	Initial	Development	Intermediary	Finale	Amount
Beginning of stage	21/05/2014	10/06/2014	11/07/2014	29/08/2015	
Period (days)	20	31	49	20	120

**Table 2.** evapotranspiration bean (*Phaseolus vulgaris* L.) on the dates and points sampled on Landsat 8 images.

Dates	DAS	ETo (mm/day)	ETc (mm/day) (FAO)	ETa (mm/day) (SAFER)	Absolute error (mm/h)	Relative error (%)
30/jun	40	2.22	1.95	1.69	0.27	13.72
16/jul	56	2.19	2.52	2.52	0.00	0.04
1/augu	73	2.77	3.19	3.21	0.03	0.87
17/augu	88	3.11	3.58	3.58	0.00	0.00
02/sept	105	2.99	2.81	2.63	0.18	6.32

with the estimation, through simple linear regression. R-squared, Pearson "r" correlation coefficient and Willmott "d" index were considered statistical indicators. Willmott index is related to the removal of the estimated values from observed values, ranging from zero for no agreement to 1 for perfect agreement, being determined by Equation 8:

$$d = 1 - \left[ \frac{\sum (P_i - O_i)^2}{\sum (|P_i - O_i| + |O_i - O|)^2} \right] \quad [8]$$

Where  $P_i$  is the estimated value;  $O_i$  is the observed value and  $O$  is the mean of the observed values. However, climatic conditions contain various weather parameter values, and research results are not applicable for other regions without specified ranges being determined for each weather parameter, even if climatic conditions are the same for both regions (Valipour, 2015).

Although FAO Penman-Monteith has been applied in various regions of the world, its application requires many parameters that are often difficult to be obtained. To this end, experimental models have been developed for potential evapotranspiration estimation using limited data (Valipour, 2014). Therefore, SAFER is an algorithm that has the advantage of applying meteorological data from different station types (agrometeorological, conventional and automatic). Algorithms are relatively recent technology advances in evaporation estimation.

## RESULTS AND DISCUSSION

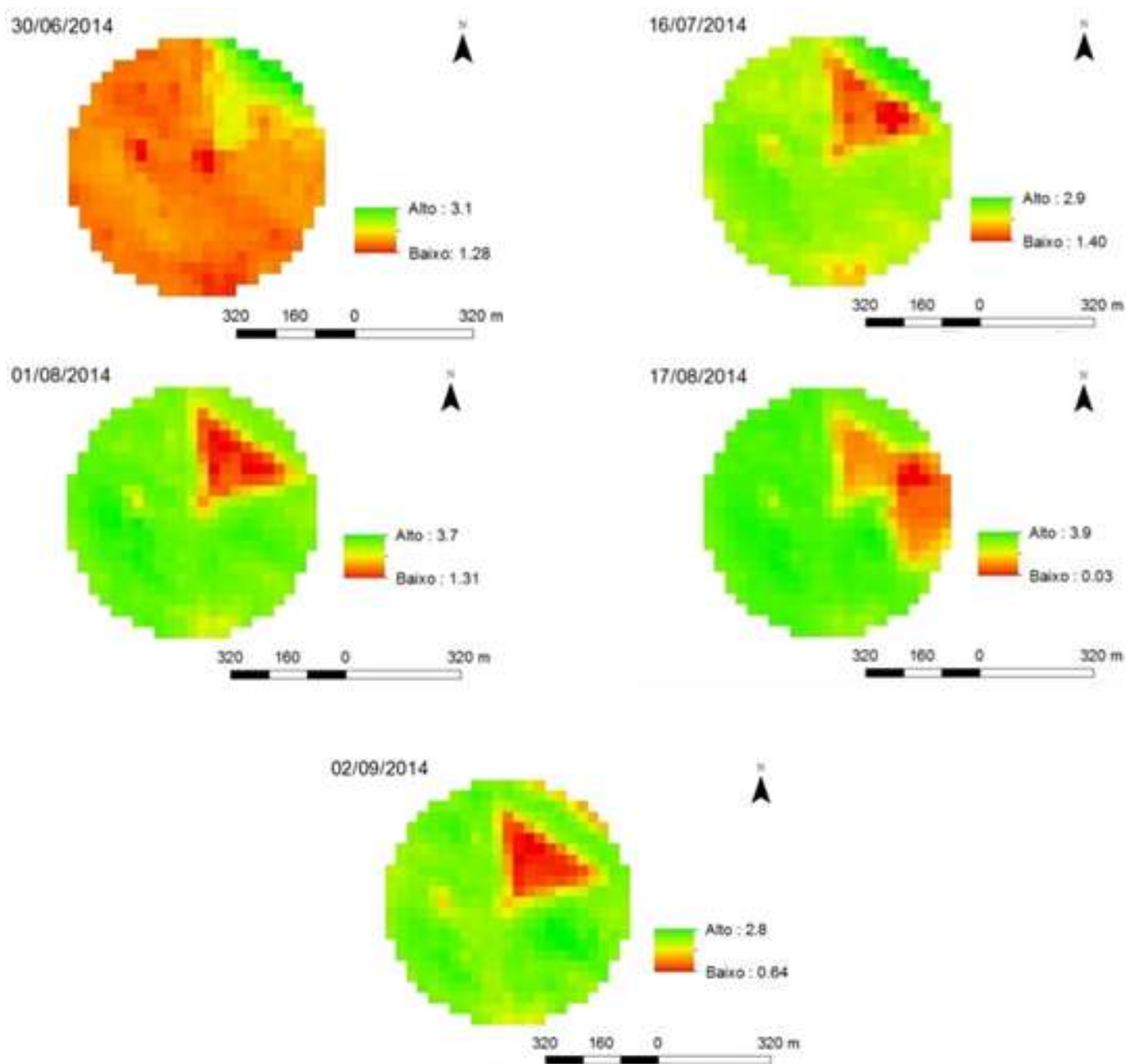
Based on the values obtained in the bean crop seeded in winter, May, and phenological differences monitoring, the duration of each crop development stage was determined, as seen in Table 1. Table 2 shows evapotranspiration reference (ETo) values, determined by conventional method (FAO-56), and actual evapotranspiration (ETa), using SAFER in five different periods throughout the crop development cycle. Data from weather stations, available by Irriger, were used as

evapotranspiration reference (Penman-Monteith). From the relative error percentage, average data variation was estimated within the 4.19% area.

ETa in the months from June to September, 2014, for a defined study area, is observed in Figure 1. Note that, depending on crop development stage, ETa values vary. However, higher values were observed at the stage where the crop was in full development. The lowest ETa values were observed in the first and last periods, due to being the closest to the beginning and the end of the development period.

When comparing ETa values estimated by SAFER with ETc values, there is great similarity between data, especially in the phases in which the crop was in full development (07.16, 08.01 and 08.17). On the first period (30.06; 40 DAS), SAFER model estimated a value below the crop evapotranspiration value obtained by the standard method due to being closer to the beginning of the development period, with relative error of 13.72%, which is higher than reference evapotranspiration, as shown in Figure 2. This can be explained by soil surface moistening frequency effect, which is caused by uncovered surface or little vegetation, or by the irrigation frequency in the area.

Approaching the final stage of the cycle (02/09), the model underestimated the ETo value (Figure 2). This effect can be explained by the fact that the crop was entering the final stage of the growing season, which is a stage characterized by senescence, and beginning the maturation process, what has direct influence in NDVI determination, making NDVI values decrease to initial levels. It is observed in Figure 2 that Pearson "r" correlation showed a value of  $r = 0.99$ , which was very close to the Willmott "d" index, 0.97, and very close to the R-squared value,  $R^2 = 0.98$ , which indicates a strong relation between the analyzed variables, meaning good results.



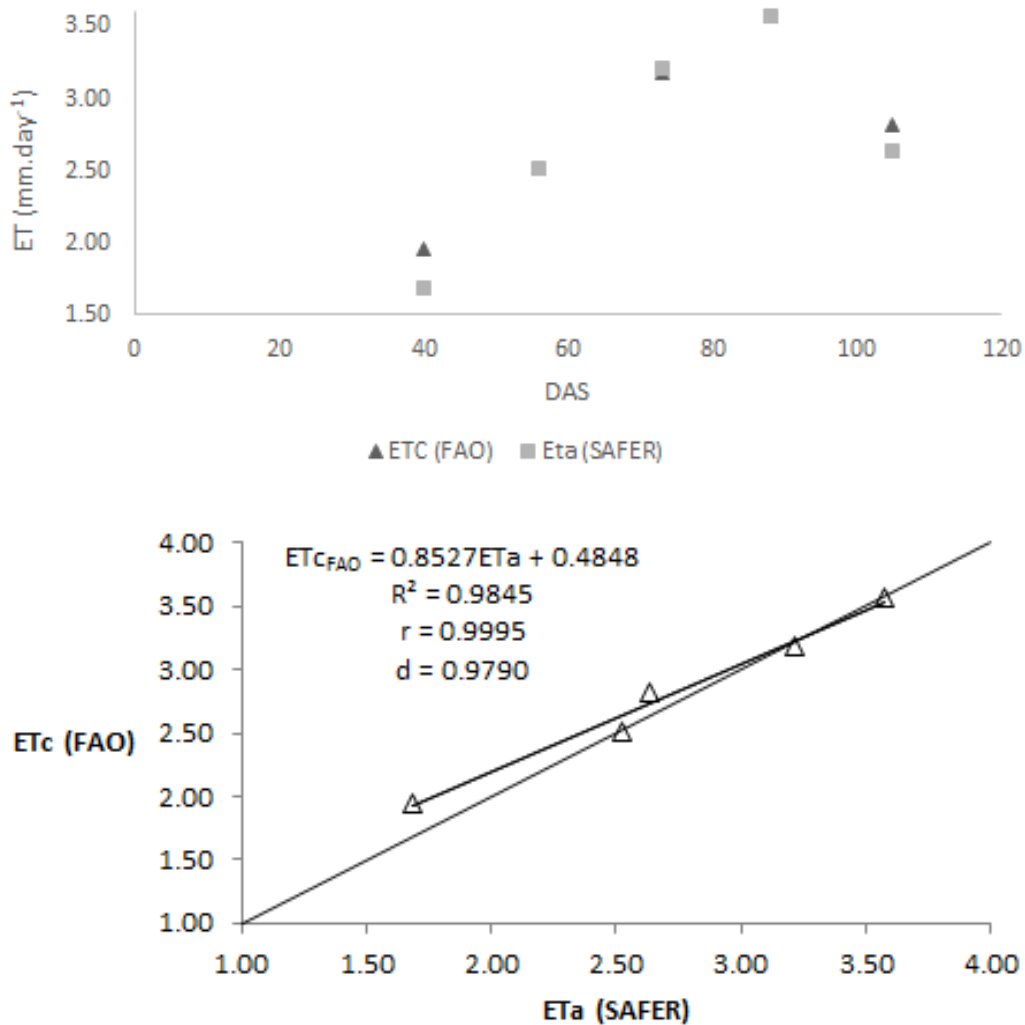
**Figure 1.** Actual evapotranspiration map for the period from June to September 2014, in the demarcated area of study.

Figure 3 illustrates the crop coefficient ( $K_c$ ) curve obtained by the SAFER model and by the standard method in the satellite passage periods. Figure 3 also analyzes standard crop coefficient and SAFER model estimation differences. Higher values were observed to the standard crop coefficient at the stage where the crop was in early development and at the end of the growth period. This can be explained because the plant surface was uncovered or with little cover. Based on the obtained

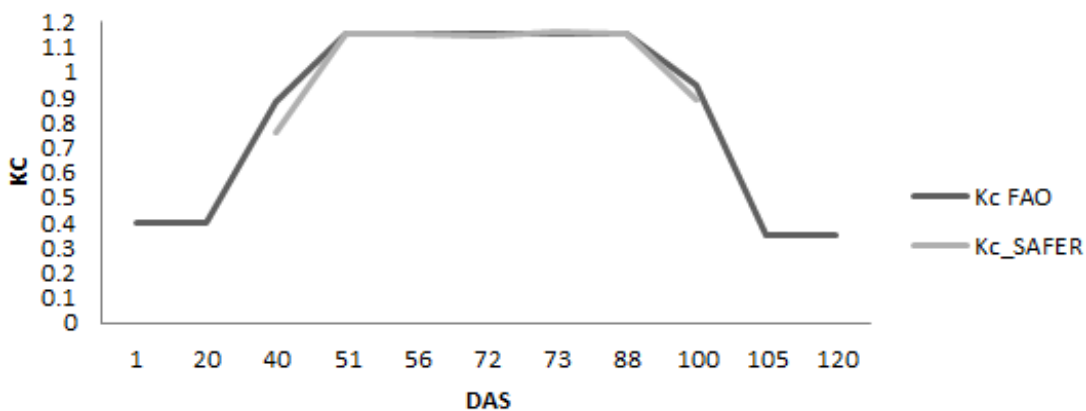
results, SAFER model was consistent in assessing water consumption and identifying potential problems in the uniformity of application of water by the irrigation system.

### Conclusion

According to the results, it is concluded that SAFER is a very promising algorithm to estimate actual crop



**Figure 2.** Comparative ET determined by standard method (FAO-56) and actual evapotranspiration (ET) using SAFER at different periods throughout the crop development cycle.



**Figure 3.** Comparison between bean crop Kc using FAO-56 report and Kc using SAFER in different periods throughout the crop development cycle.

evapotranspiration, highlighting the possibility of monitoring the timeline of large irrigation areas. Therefore, useful information to assist the producer in irrigation system planning and management decision-making can be obtained by SAFER.

### Conflict of interest

The authors have not declared any conflict of interest

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## Full Length Research Paper

# Performance of populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on different varieties of maize

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Post-harvest losses of maize due to storage insect pest such as the maize weevil, have been recognized as an increasingly important problem in the world. A study was conducted with the objectives to determine the performance of *Sitophilus zeamais* populations originating from the five Brazilian regions in five varieties of maize and to correlate these variables with the enzymatic activity of insects and nutritional components of the grain. The grain of maize hybrids 30F53H, 30K64H, 30F35H, DKB390 and “crioulo” (not commercial) maize produced in the microregion of Alto M dio Gurgu ia in Piau , was infested with 25 unsexed insects of *S. zeamais* coming from Bom Jesus, PI; Canarana, MT; Volta Redonda, RJ; Cacoal, RO; and Palmeira das Miss es, RS. At the end of 120 days enzyme assays of amylase, lipase and protease from the insects, as well as nutritional analyses of the maize grains were performed. The hybrid with the greatest mass of grain consumption was 30F53H, and insects fed on this achieved the highest growth, followed by 30K64H, DKB390, 30F35H and the “crioulo” with the lowest loss of grain mass and hence the least insect growth. For the data of mass consumed by *S. zeamais*, the populations of Bom Jesus and Canarana were the ones that ate the most and Palmeira das Miss es had the lowest consumption. The population from Bom Jesus, PI was the one that consumed the most grain mass, due to its adaptation to maize from the region. The “crioulo” maize was the one that presented the best indicators of resistance to attack by *S. zeamais*.

**Key words:** Digestive enzymes, maize weevil, host plant resistance to insects, stored grain.

## INTRODUCTION

Maize, *Zea mays* L., is an important crop ranking second in world grain production only preceded by wheat. Maize

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production and distribution is a cosmopolitan (Makate, 2010) and it is an important component of agriculture and food systems all over the world. The crop is versatile in its uses and environmental adaptation. It is consumed all over the world both by human being and animals. Maize grains find themselves in many industries to be processed in to various food and industrial products of multi-purpose functions. The infestation of maize by maize weevil commences in the field but the lion share of the damage to maize grains by maize weevil is done during storage period. Such damaged grains have reduced nutritional values, low percent germination and reduced weight and market values (Trematerra et al., 2013). Its control is accomplished primarily with insecticides (Miranda et al., 1995; Ferrari-Filho et al., 2011).

Consumers have demanded, from producers and companies, grain and products free of toxic residues. Given this situation, scientists in the search for methods to control insects that are less harmful to the environment and offer less risk to human and animal health have intensified their research in the development of insect-resistant plants, being a more specific and efficient method of control (Keba and sori, 2013).

Grains that express inhibitors of insect digestive enzymes, especially of  $\alpha$ -amylases, represent a promising tool for the control of stored product pests because these insects live on a diet rich in polysaccharides and depend on the effectiveness of their amylases for survival (Pueyo et al., 1995; Ishimoto et al., 1996; Mendiola-Olaya et al., 2000).

It is important to compare the development of different populations of the weevil in different maize varieties, because the large differences between populations of the maize weevil are the result of the discontinuous nature of grain storage and also the management of these insect pests within the grain storage units. The distribution of these units together with their management probably accentuates the seasonal cycles of these populations and enables the rapid establishment of new populations from a small number of individuals (Tran and Credland, 1995; Guedes et al., 1997; Keba and Sori, 2013). Within the context of integrated pest management, knowing the behavioral and physiological differences among these populations is an important control tool. The objective of this work was to evaluate the performance of populations of *S. zeamais* coming from the five Brazilian regions in five varieties of maize and to correlate these variables with the enzymatic activity of the insects and nutritional components of the grain.

## MATERIALS AND METHODS

### Varieties of maize

The maize grain used was produced in the region of Alto Médio Gurguéia, Piauí, in the 2010/2011 harvest. The hybrids 30F53H, 30K64H, 30F35H, DKB390 (not Bt) and the "crioulo" variety from

the region of Santa Luz, PI were utilized. These were not subjected to any form of phytosanitary treatment, being previously selected, eliminating impurities and defective grains that could compromise the experiment. Then, they were stored for 10 days under refrigeration at  $-10^{\circ}\text{C}$  to eliminate insects in their various stages of development. After this period, 10 kg of grain was thawed and dried naturally in plastic containers, sealed with organza type fabric and kept at 13% humidity.

### Initial population of *Sitophilus zeamais*

The populations of *S. zeamais* used at all stages of the experiments were collected in different storage units in Brazil: Bom Jesus, IP; Canarana, MT; Volta Redonda, RJ; Cacoal, RO; and Palmeira das Missões, RS. The populations were bred from maize samples collected in the field and kept in 2 L plastic containers covered with an organza-type fabric. The experiment was maintained in a laboratory at room temperature ( $25$  to  $27^{\circ}\text{C}$ ) and 40 to 60% RH and photofase of 14 h.

### Population growth and loss of grain mass

The experiment was conducted in a completely randomized design with five varieties of maize, five populations of *S. zeamais*, of different geographical localities, and three replications. For each treatment 600 ml plastic containers containing 250 g of maize were used, these being infested with 25 unsexed *S. zeamais* insects aged 1 to 10 days. The number of live and dead insects and grain and insect weight lose was evaluated at 120 days. At the end of each period, the grain was sieved to separate the maize grain from the insects and their droppings. The numbers of live and dead insects and grain mass consumed were recorded. At 120 days, after data collection, live insects from each treatment were frozen at  $-10^{\circ}\text{C}$ , until needed for bioassays to perform enzyme bioassays and measure protein.

### Enzymatic activity of the homogenate of *S. zeamais*

Random samples of 90 non-sexed adult insects of each population of *S. zeamais* fed different maize varieties in the population growth experiment (120 days on feed) were immersed in 1.5% KCl solution (m/v), macerated in a porcelain crucible and mixed with 10.0 ml of water at pH 3.0. The extract was filtered through cheesecloth and centrifuged at 3500 rpm for 30 min. The precipitate was discarded and aliquots of the supernatant were taken for determination of total protein concentration and specific activity of the enzymes amylase, lipase and total protease. The concentration of protein in the enzyme extracts was determined by the Warburg and Christian (1941) method for determination of specific activity.

For amylase assay the BIOCLIN K003 kit (Quibasa - Basic Química Ltda, Belo Horizonte, Minas Gerais, Brazil) was used, containing substrate (starch +  $0.4\text{ g L}^{-1}$  phosphate buffer (pH 7.0)  $100\text{ mmol L}^{-1}$  + stabilizers and preservatives) and color reagent ( $50\text{ mmol iodine L}^{-1}$  + stabilizer). This reaction is based on fixed time kinetics (Caraway, 1959) modified. The enzyme extract was incubated with the starch substrate and the absorbance reading was taken at a wavelength of 660 nm.

For the lipase assay the BIOCLIN K025 kit (Basic Química Ltda, Belo Horizonte, Minas Gerais, Brazil Quibasa) was used, containing buffer (methane hydroxymethylamino  $100\text{ mmol L}^{-1}$ ) (pH 8.5), enzyme inhibitor (phenylmethyl sulfonyl fluoride  $8\text{ mmol L}^{-1}$  + solubilizer), color reagent ( $3\text{ mmol ditionitrabenzóico acid L}^{-1}$  + sodium acetate  $100\text{ mmol L}^{-1}$  + stabilizer), substrate (ditiopropanol tributryrate  $20\text{ mmol L}^{-1}$  + surfactant) and acetone. The absorbance reading was taken at a wavelength of 410 nm.

To obtain the graph of enzyme kinetics, according to Michaelis-Menten for the enzymes amylase and lipase, reactions were performed with aliquots of substrate from 25 to 200  $\mu\text{l}$  and 10 to 80  $\mu\text{l}$ , respectively. The absorbance values from each aliquot were converted into enzyme speed values (amylase unit dL mg protein, for amylase and IU  $\text{mg}^{-1}$  protein for lipase).

For the total protease activity azocasein was used as substrate at 2% (w/v) in 0.1 M Tris-HCl buffer, pH 8.0, 37°C according to the method described by Tomarelli et al. (1949). The reaction mixture consists of 250  $\mu\text{l}$  of substrate and 300  $\mu\text{l}$  of enzyme extract, incubated at 37°C for 30 min. Soon after, the reaction was stopped with 1.2 ml of trichloroacetic acid (TCA) at 10% (v/v) and allowed to stand for 15 min on ice. Before reading the absorbance at 440 nm, 1.4 ml of 1.0 M NaOH was added. All assays were performed with four replications.

### Analysis of nutritional grains

Samples of 100 g of each grain (free infestation), variety were ground separately, and aliquots of these samples underwent moisture determination by weight loss, in an oven at 105°C, until constant weight; ashes were obtained by incineration of the material in an oven at 550°C. The crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude fiber (CF) were determined according to the methodology described by Silva and Queiroz (2002). The ether extract was determined by hot extraction with the use of petroleum ether as organic solvent (Silva and Queiroz, 2002). The experimental design was completely randomized with four replications. The results were expressed on a dry basis. Analysis of starch was performed using the methodology of Hendrix (1993) in the same experimental scheme previously adopted.

### Statistical analysis

Analysis of variance was performed on the completely randomized factorial design with three replications for the characteristics of population growth and loss of grain mass and four replications for enzymatic activity, for each response variable. The F-test ( $p \leq 0.05$ ) was applied and, when a difference was observed for the interaction, an SNK comparison of means test was performed ( $p \leq 0.05$ ). For the nutritional analyses of grain we performed analysis of variance for the response variables, with standard procedure for a completely randomized design with three replications. When a significant difference was detected a means test was performed. Subsequently we performed Pearson correlation analysis on all response variables. All analyses were performed with the aid of the SAS computer program (SAS, 2002) by PROC GLM and PROC CORR. The curved non-linear regressions of Michaelis Menten type were plotted using the Sigma Plot program (SPSS®, 2000).

## RESULTS AND DISCUSSION

The results demonstrated there is a difference in the growth of populations of *S. zeamais* on different maize varieties. There was an interaction between populations and varieties, and differentiated behavior of populations was seen on different varieties. The no-choice test was conducted to check for differences in some biological characteristics of *S. zeamais* between insect populations and tested varieties of maize and their interactions. For the characters population growth, insect mortality and

loss of grain mass there was a difference by F-test for all analyzed factors and their interactions.

According to the data presented in Table 1, there was greater population growth of *S. zeamais* on the 30F53H variety, except for the population from Volta Redonda, RJ (215.33). Among the populations with higher growth, Bom Jesus, PI distinguished itself with an average of 701.66 insects on the 30F53H variety. A similar result was found by Caneppele et al. (2003), who evaluated the increase in infestation by 50 insects of the species *S. zeamais* in maize grain stored for 120 days, observing an infestation of 899 insects at the end of this timeframe.

According to Toscano et al. (1999), who evaluated the resistance of 30 maize genotypes to attack by *S. zeamais*, the highest rates of growth are associated with genotype susceptibility to insect attack. Thus, among the varieties of maize subjected to attack by *S. zeamais*, representing the five regions of Brazil, a difference was observed. The most susceptible variety was the single transgenic hybrid 30F53H, on which *S. zeamais* collected in Bom Jesus, PI and Canarana, MT, from the Northeast and Midwest regions respectively, had higher population growth. The maize genotype with the greatest resistance was the “crioulo” maize. It deserves mentioning that all the simple hybrids tested in this experiment were more attacked than the landrace cultivar, and that among the hybrids, the 30F35H was the most resistant to damage caused by *S. zeamais*.

Analyzing mortality data (Table 1), it is observed that the highest cumulative mortality occurred with the population of Canarana, MT on the variety DKB390 (228 individuals). The lowest cumulative mortalities were found in the population from Palmeira das Missões that had the lowest growth and less loss of grain mass. The hybrid 30K64H had a lower average mortality of populations, and 30F35H had high mortality and low insect growth.

The hybrid that had the greatest loss of grain mass was 30F53H, which was also the one that had the greatest growth, followed by 30K64H, DKB390, 30F35H and the “crioulo” maize with the lowest mass loss and hence lower growth. For the mass consumption data by *S. zeamais* in this study, it was seen that the populations of Bom Jesus and Canarana were the ones that caused the greatest losses, followed by Volta Redonda and Cacoal, and there were lower losses for Palmeira das Missões (14, 14, 10, 10 and 9%, respectively). These data are similar to those of Caneppele et al. (2003) who studied the correlation between the level of *S. zeamais* infestation and the storage quality of maize grains. They noted an increase in weight loss of these grains, as time and the number of insects in contact with the maize grains increased. The authors observed weight losses of 15% in the 120 days period, when 50 insects were used in the initial infestation. Antunes et al. (2011) found losses of 17% after 120 days of storage of 600 g of maize with an initial infestation of 150 insects. In agreement with other authors, Marsaro-Junior et al. (2007) found greater



**Table 2.** Nutritional analysis of grain of five varieties of maize: dry mass (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ashes, crude fiber (CF), starch and lipids (mean).

Variety	Percentage							
	DM	CP	NDF	ADF	Ashes	CF	Starch	Lipids
30F53H	92.54 <sup>a</sup>	10.42 <sup>a</sup>	32.41 <sup>b</sup>	8.05 <sup>b</sup>	1.08 <sup>a</sup>	2.75 <sup>a</sup>	53.20 <sup>ab</sup>	3.51 <sup>c</sup>
30K64H	92.44 <sup>a</sup>	8.72 <sup>b</sup>	24.16 <sup>c</sup>	7.36 <sup>b</sup>	1.72 <sup>a</sup>	2.24 <sup>a</sup>	52.66 <sup>ab</sup>	4.75 <sup>b</sup>
“Crioulo”	92.54 <sup>a</sup>	8.46 <sup>b</sup>	35.99 <sup>a</sup>	9.75 <sup>a</sup>	1.26 <sup>a</sup>	2.57 <sup>a</sup>	53.82 <sup>ab</sup>	3.80 <sup>c</sup>
30F35H	92.18 <sup>a</sup>	9.64 <sup>ab</sup>	30.90 <sup>b</sup>	6.63 <sup>b</sup>	1.06 <sup>a</sup>	2.43 <sup>a</sup>	54.50 <sup>a</sup>	5.32 <sup>a</sup>
DKB390	91.69 <sup>a</sup>	8.63 <sup>b</sup>	34.57 <sup>a</sup>	7.67 <sup>b</sup>	0.97 <sup>a</sup>	2.43 <sup>a</sup>	52.10 <sup>b</sup>	3.69 <sup>c</sup>

\*Means followed by the same letter in a column (varieties of maize) do not differ by SNK test at 5% probability.

five regions of Brazil fed the five maize varieties (Table 3).

The Michaelis-Menten equation model (Figures 1 and 2) was derived to consider the kinetic properties of the enzymes. The Michaelis-Menten constant (KM) and the maximum reaction rate (Vmax) are the kinetic parameters determined by the hyperbola. The first, for example, KM, represents the substrate concentration at which the velocity reaches half of the maximum speed and in its simplest form and is a measure of the affinity of the enzyme for the substrate. The kinetic parameter Vmax is reached when all the active sites are filled with substrate molecules. Both lipase and amylase for all treatments followed the Michaelis-Menten curves, but for lipase, in the 30F53H, landrace and DKB390 varieties, except in the Canarana, MT population which showed greater lipase activity, the remaining populations showed lower speed of the enzyme reaction due to a lower amount of lipase among the insect homogenate protein (Figure 2).

In insects, the fat body is the major site of synthesis and storage of carbohydrates, lipids and proteins, which are readily used as an energy source for many different activities (Chown and Nicolson, 2004). The enzyme amylase is responsible for hydrolysis of  $\alpha$ -1.4 glycosidic linkages within starch and related polysaccharides (Lehninger et al., 2000; Zeng and Cohen, 2000). Many organisms, including insects that are serious pests in stored grains, live on a diet rich in polysaccharides and depend on the effectiveness of their amylases to survive in metabolic terms (Mendiola-Olaya et al., 2000). Moreover, this enzyme plays a central role in carbohydrate metabolism causing organisms that have a diet rich in starch to depend on the effectiveness of their amylases for survival (Titarenko and Chrispeels, 2000). This is the case with insects that are serious agricultural pests and consume plant organs rich in starch such as seeds and roots (Titarenko and Chrispeels, 2000).

The populations studied showed differences in the mobilization of starch in the different varieties studied. The high amylase activity in the Palmeira das Missões and Cacoal populations suggests greater importance of this enzyme in digestive metabolism as a source of large amounts of carbohydrate for accumulation in fat bodies of

this same population. The digested starch appears to be used only for storage and not to supply sugar for energy metabolism.

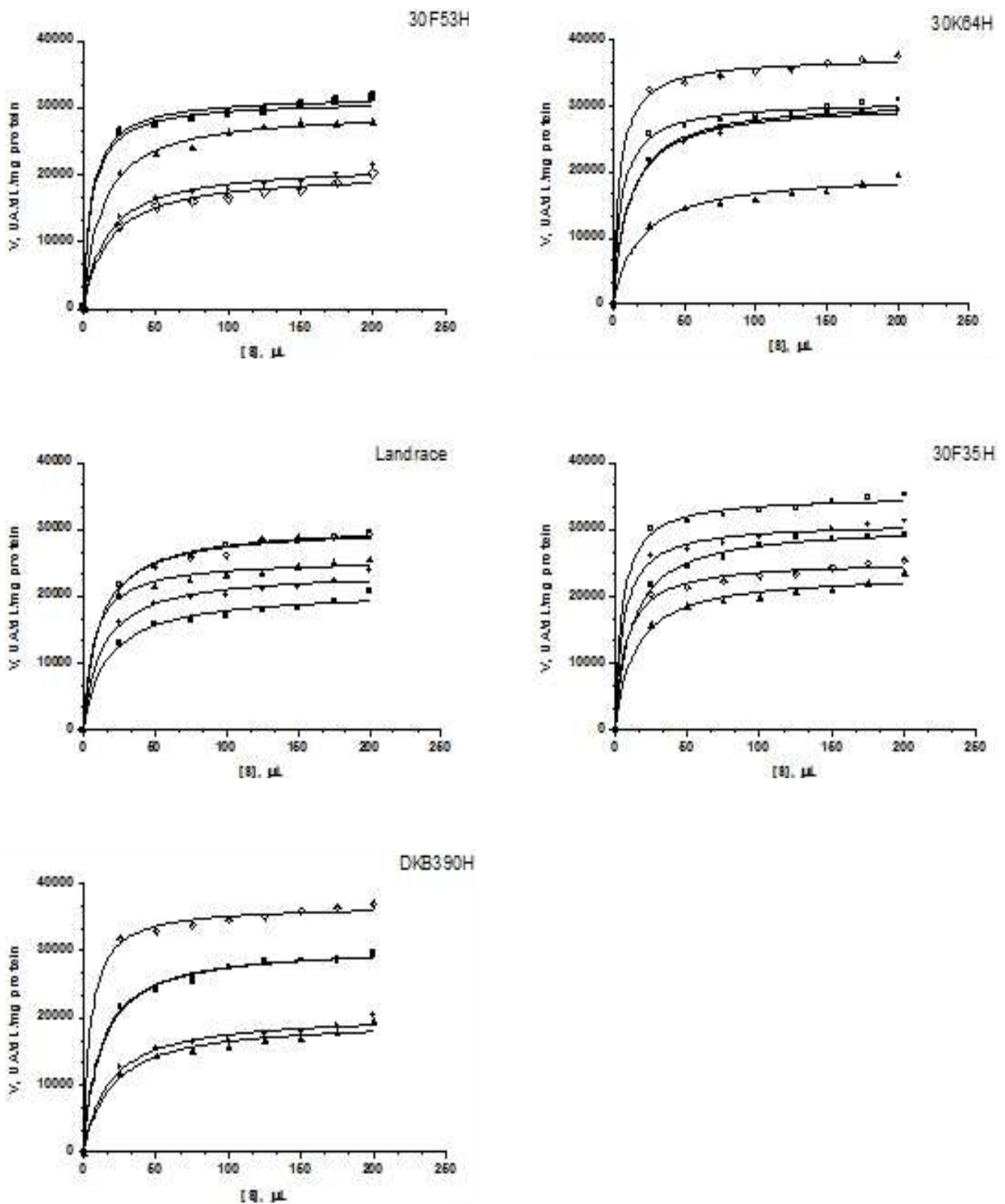
The levels of lipids in the grains showed a positive correlation with amylase and lipase in insects and a negative correlation with crude fiber, crude protein and mortality, but did not correlate with mass consumed and emergence (Table 4). In Marsaro-Junior et al. (2005), of the nutritional parameters evaluated, lipids were the ones that most influenced the resistance of hybrids to attack by *S. zeamais*, since lipid content was positively correlated with the life cycle of the pest ( $r = 0.46$ ,  $p < 0.05$ ), indicating that increasing the lipid content in the grain leads to an increase of the biological cycle, that consequently provides a lower number of generations produced by *S. zeamais*, resulting in less weight loss of grain dry matter.

Canepelle et al. (2003) also found a positive correlation between the number of insects and loss of grain weight ( $r = 0.95$ ,  $p < 0.001$ ) on evaluating the correlation between level of infestation of *S. zeamais* and the quality of stored grain, similar to that found in the present study (Table 4). Regarding access and utilization of food, it was verified by enzyme activity (amylase and lipase), that after the food is ingested, there was no difference in responses to a given variety, as indicative of resistance associated factors. There are different answers regarding growth in relation to maize variety, this can be considered in handling maize varieties resistant to attack by *S. zeamais*. These results allow us to conclude that the factors of resistance of maize are substances that will be assimilated subsequently, inhibiting population growth as was observed in the present work with the grain of landrace maize, without any genetic manipulation.

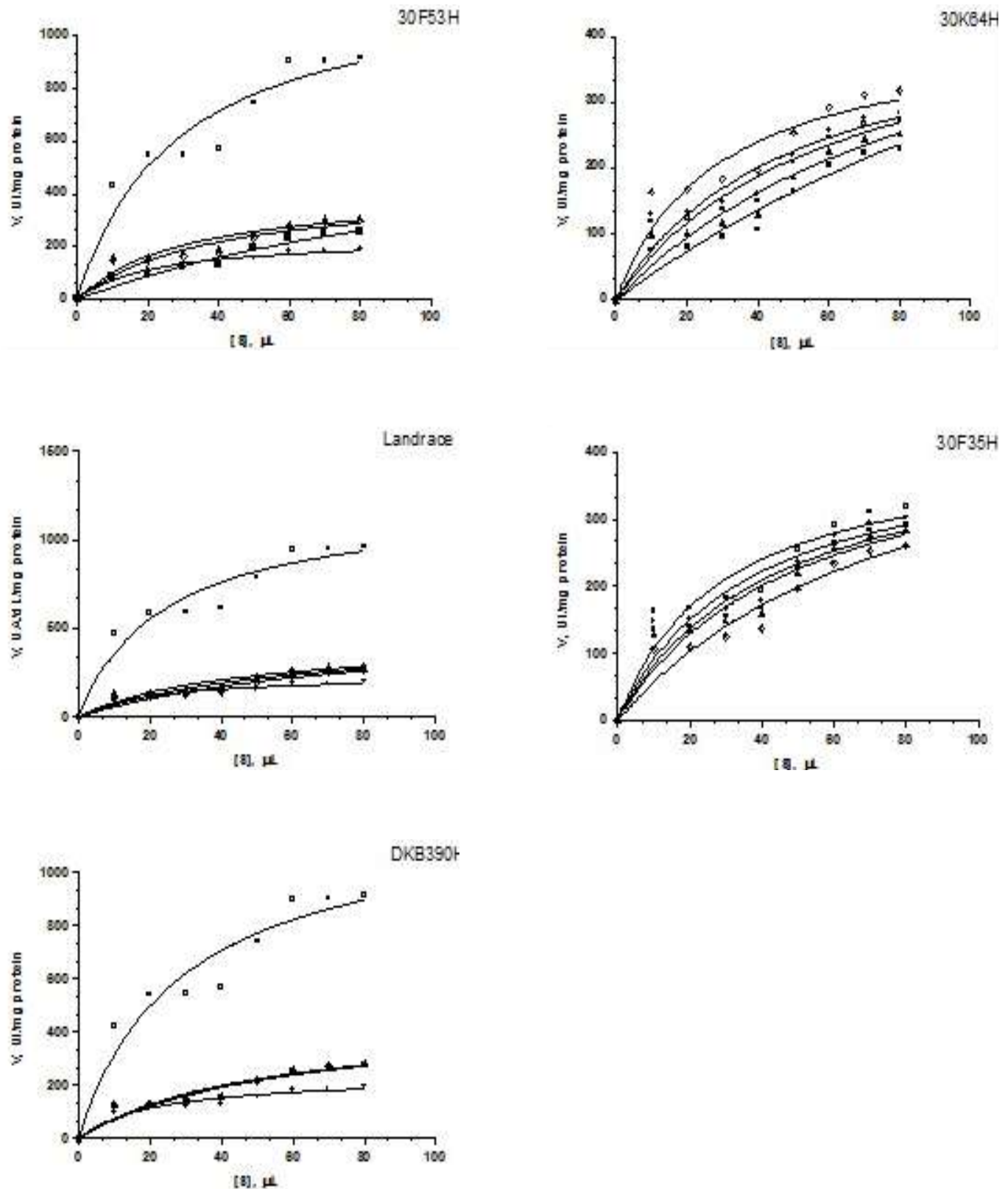
According to the *S. zeamais* population growth data and enzymatic activities, it can be inferred that the resistance or susceptibility of grain to pest attack is related to physical and chemical constituents of the grain than the assimilation of carbohydrates and subsequent storage in fat tissue, as can be verified by analyzing the results of enzyme activity (amylase, lipase and protease) of insects fed on different maize varieties.

Populations from different regions show different





**Figure 1.** Effect of substrate concentration on the rate of the reaction catalyzed by AMYLASE homogenate of *Sitophilus zeamais* (individuals collected in Bom Jesus ● PI; ■ Cacoal RO; □ Canarana MT; ▲ Volta Redonda RJ; ◇ Palmeira das Missões RS) fed different maize varieties 30F53H; 30K64H; Landrace; 30F35H; DKB390 determined by Michaelis Menten curve ( $p < 0.001$ ,  $R^2 > 0.95$ ).



**Figure 2.** Effect of substrate concentration on the rate of the reaction catalyzed by LIPASE homogenate *Sitophilus zeamais* (individuals collected in Bom Jesus ● PI; ■ Cacoal RO; □ Canarana MT; ▲ Volta Redonda RJ; ◇ Palmeira das Missões RS) fed different maize varieties 30F53H; 30D64H; Landrace; 30F35H; DKB390 determined by Michaelis Menten curve ( $p < 0.001$ ,  $R^2 > 0.95$ ).

**Table 4.** Correlation coefficients among twelve variables considering the resistance of five maize varieties to five populations of *S. zeamais*.

Variables	Amylase	Lipase	Protease	DM	CF	Starch	Lipids	CP	CM	Mort	NI
Amylase											
Lipase	0.76**										
Protease	0.43**	0.26*									
MS	-0.52**	-0.47**	-0.44**								
FB	-0.59**	-0.65**	-0.06 <sup>NS</sup>	0.23*							
Starch	-0.51**	-0.48**	-0.31*	0.43**	0.21*						
Lipids	0.48**	0.58**	-0.18 <sup>NS</sup>	-0.06 <sup>NS</sup>	-0.66**	-0.10 <sup>NS</sup>					
CP	-0.49**	-0.54**	-0.13 <sup>NS</sup>	0.40**	0.53**	0 <sup>NS</sup>	-0.52**				
CM	-0.06 <sup>NS</sup>	0 <sup>NS</sup>	-0.02 <sup>NS</sup>	0.05 <sup>NS</sup>	0.03 <sup>NS</sup>	-0.09 <sup>NS</sup>	0.12 <sup>NS</sup>	0.26*			
Mort	-0.01 <sup>NS</sup>	-0.03 <sup>NS</sup>	0.44 <sup>NS</sup>	-0.30 <sup>NS</sup>	0.10 <sup>NS</sup>	-0.15 <sup>NS</sup>	-0.30**	0.06 <sup>NS</sup>	0.30**		
NI	0.07 <sup>NS</sup>	0.13 <sup>NS</sup>	0.25 <sup>NS</sup>	-0.14 <sup>NS</sup>	0 <sup>NS</sup>	-0.19 <sup>NS</sup>	0 <sup>NS</sup>	0.15 <sup>NS</sup>	0.69**	0.65**	
Emer	0.06 <sup>NS</sup>	0.12 <sup>NS</sup>	0.27 <sup>NS</sup>	-0.14 <sup>NS</sup>	0.02 <sup>NS</sup>	-0.20*	0.03 <sup>NS</sup>	0.18 <sup>NS</sup>	0.69**	0.68**	0.99**

DM – dry mass, CF – crude fiber, CP – crude protein, CM – consumed mass, Mort – mortality, NI – number de insects, Emer – emergence of insects. \*\* , \* and NS, significant at 1%, 5% and not significant, respectively.

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*Full Length Research Paper*

# Altered climate and livelihood of farming families in Niger Delta region of Nigeria

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This study focused on how altered climate has affected the livelihood of the farmer and the farming families in Niger Delta region of Nigeria. The study adopted descriptive survey research design. This study had two research questions and two hypotheses. The population for the study was 246,807 made up of registered farmers. Proportionate stratified random sampling technique was used to select a sample size of 4,936 as respondents. Structured questionnaire was used to collect data. The instrument was face validated by three experts. Cronbach alpha method was used to determine the internal consistency of the questionnaire items which yielded a coefficient of 0.81. Mean, standard deviation, and t-test were used for data analysis. The findings of the study revealed that altered climate have adversely influenced the livelihood and living conditions of the farmer and the farming families in the area studied. Findings further revealed that the altered climate has led to increased poverty level and raised cost of production (input and labour cost), thus affecting farmers hitherto coordinated livelihood. Adoption of local adaptation approaches will help the farmers survive.

**Key words:** Farmer, flooding, sea level rise, rainfall, sustainable agriculture.

## INTRODUCTION

The importance of agriculture to humans and the society have been continually lauded to include sources of revenue for governments at various levels and as a means of livelihood by providing employment for farmers, marketers, and processors of agricultural products. In Nigeria, agriculture engages over 70% of the labour force and contributes about 40% to Gross Domestic Product (GDP) (FMARD, 2012). It provides food for the teeming population, feed for animals and raw materials for various industries. However, it is one of the most climate

sensitive economic sectors as a change in climate bears a direct effect on agriculture. Altered climate, generally termed climate change has been an international issue affecting various agricultural production processes, including the producers: the farmer and his families. Altered climate is the total or average variation of the atmosphere over a period of time, usually from decades to many years in a location which can be caused by processes internal to the earth, external forces from space or human activities (Lobell et al., 2008).

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Altered climate has often been referred to as the noticeable change in climate that persists for an extended period, typically decades or longer (IPCC, 2007). Ikehi and Zimoghen (2015) described altered climate as the variation in the statistical distribution of average weather conditions over a prolonged period of time leading to adverse effects on the farmer and the farming families in any region of the world, such as the Niger Delta region.

The Niger Delta region of Nigeria is densely populated and occupies about 12% of the total land mass of Nigeria with a land area of about 70,000 km<sup>2</sup> out of which 2,370 km<sup>2</sup> consist of rivers, creeks, and estuaries, while stagnant swamp covers about 8,600 km<sup>2</sup> (Ugolor, 2004). The region is divided into drier landward part where crop farming is the major agricultural activity and the seaward part (riverine and swampy area) which is characterized by extensive creeks and water bodies, where fishing and aquaculture replaces crop farming as the dominant aspect of the rural economy (Aweto, 2011). The economic activities of the communities in the region are either land-based or water-based to include collection and processing of palm fruits, crop, and animal farming, fishing and fish farming, forest resources utilization (such as game, raffia), general farming and trading of agricultural goods (Rosemary et al., 2012). The region is endowed with great potentials for high productive and profitable agricultural practice and occupies greater area of Nigeria's most fertile land suitable for the production of crops such as cassava, palm tree, rubber, yam, and many other crops while the availability of water bodies make feasible aquaculture like fish farming (Fapojuwo et al., 2012; Abisola, 2013). The major occupation in the region is farming thus an altered climate affects the farmer and his farming family.

The main effect of altered climate is the increasing average temperature which causes a variety of secondary effects (IPCC, 2007). The secondary effects include changes in patterns of precipitation affecting rainfall, rising sea levels, altered patterns of agriculture, increased extreme weather events, expansion of the range of tropical diseases, among others (IPCC, 2007; Ogundele, 2012; Ikehi et al., 2014). These secondary effects have affected the world in varying degree of impacts in different regions. As ecosystem is being affected, people especially the indigent who cannot cope with the drastic changes may have to migrate in search for fairer and better opportunities. Estimates of future "climate change migrants" range from 200 million to 1 billion by 2050 (Myers, 2005). Africa is one of the most vulnerable continents to face climate change, because of multiple existing stress and low adaptive capacity (Mimura et al., 2007). According to these authors, sea level rise is projected to worsen inundation, storm surge, erosion, and other coastal hazards in the continent and these effects would threaten vital infrastructure, settlements, and facilities that support the livelihood of isolated communities. In many African countries, other

factors already threatening human health such as malaria may increase as altered climate conditions could favour the proliferation of carrier agents (Boko et al., 2007). As estimated, by 2020, between 75 and 250 million people are projected to be exposed to increased water stress and by 2050, between 350 and 600 million people in Africa are projected to experience increased water stress due to altered climate (Boko et al., 2007). Niger Delta like other deltas in the continent is recognized as being vulnerable to the effects of altered climate due to its low-lying area.

Flooding in the region (between July and October 2012) forced rivers to overflow their banks and submerged hundreds of thousands of acres of farmland (Hassan, 2012). Besides the destruction of buildings and lives, floods blocked transportation routes in the region. The cost of managing the land for cultivation, cure for disease and pest control in animal, crop, and fish production has been on the rise as a result of altered climate bearing direct effects on the social and economic wellbeing of farmers. The wellbeing of the farmer and his farming families is as important as the production process and the agricultural produce and climate change affects not just produces but also the farmer and the farming families as well as the environment they dwell. Leading to decline in performance, thus reduced yields. Sea level rise in the region could increase the emergence of health-related hazards for the farmer and his family.

Variation in temperature and humidity alters pest and disease movement and favours the risk of invasion. Frequent natural disasters like floods, ocean and storm surges damage sources of livelihood and also causes harm to farmland, post-harvest activities, life, and property (Uyigue and Agho, 2007; Idowu et al., 2011). Extreme storm events are likely to increase failure of floodplain protection as well as damage urban drainage and sewage systems (Apata, 2010). Increased heat causes discomfort for the farming family, while intense heat wave leads to electricity blackouts (Boko et al., 2007). Though some farming families in the region still engaged in farming, they work more with little in return. Their farming and fishing have been impaired in recent times by the deplorable state of the environment as a result of altered climate (Uyigue and Agho, 2007). According to the authors, due to the degradation of their immediate environment, the local farmer can no longer engage in sustainable production leading to low economic capacity of the already indigent farmer. The change in climate affects crops, livestock, forestry, and fishery in various ways invariably impacting on the livelihood of the farmer and his families. Many people in the Niger Delta whose source of livelihood once depended on natural sectors such as, farming and fishing are now changing their responsible means of livelihood. Change in the farming occupation will have adverse effects on agricultural sector in the region and the nation as a whole. Continued degradation of land and water as

a result of altered climate in the region will affect the major agricultural produce in the region, thus increasing hardship for the farmer and his family.

The farmers are complaining of drastic changes they could not comprehend as their previous knowledge could not serve them competently. Thus, it became imperative to empirically document the observations and plight in order to suggest suitable approaches to restore their hitherto productive livelihood. Further, it is also necessary to know if both male and female farmers have the same complain. This study thus poses the following research question and hypotheses.

### Research question

1. What are the effects of altered climate on the farming profession in the Niger Delta region?
2. What are the effects of the altered climate on the living conditions of the farmer and the farming families in the Niger Delta region of Nigeria?

### Hypotheses

H<sub>01</sub>: There is no significant difference in the mean ratings of male and female farmers on the effects of altered climate on the farming profession in the Niger Delta region.

H<sub>02</sub>: There is no significant difference in the mean ratings of male and female farmers on the effects of altered climate on the living conditions of the farmer and the farming families in the Niger Delta region of Nigeria

### MATERIALS AND METHODS

The study adopted descriptive survey research design and was carried out in the Niger Delta region of Nigeria. The region located in the Southern part of Nigeria is made up of 9 states, namely, Abia, Akwa-Ibom, Bayelsa, Cross River, Delta, Edo, Imo, Ondo, and River. The population for the study was 246,807, made up of 67,551 and 179,256 registered farmers in Bayelsa and Delta states, respectively. Among the 9 Niger Delta states, Bayelsa and Delta were the most affected ones with natural disaster, such as flood submerging and destroying farmlands in 6 out of 8 and 18 out of 25 local government areas in the Bayelsa and Delta states, respectively (NEMA, 2012). The farmers in the sampled states were involved in the study, because they have experienced in providing firsthand information for answering the research questions. Proportionate random sampling technique was used to select 2% (4,936) of the farmers from each local government areas in both states. The instrument for data collection was a structured questionnaire developed from literature reviewed for the study. The questionnaire was divided into two sections, each section corresponding to the research questions of the study. Section 1 had twenty-one (21) items while section 2 had sixteen (16) items. Each item in the questionnaire had a four point response options of High Effect (HE), Moderate Effect (ME), Low Effect (LE), and No Effect (NE) (weighted 4, 3, 2, and 1, respectively). Three experts validated

the instruments: one from Agricultural Development Programme (ADP) at Delta State Ministry of Agriculture and Natural Resources and two lecturers from the Department of Agricultural and Bioresources Education, University of Nigeria, Nsukka. The reliability of the questionnaire instrument was established using Cronbach alpha method and a co-efficient of 0.81 was obtained. To determine the reliability index, 54 copies of the questionnaire were pre-administered on both male and female farmers in Edo State. These farmers were not part of the studied population. The completed copies of questionnaire were retrieved and analyzed employing the Cronbach alpha method. The researchers with the help of 5 research assistances administered and collected the completed questionnaire. The respondents were contacted physically and the questionnaire was administered to both gender, randomly. Out of the 4,936 copies of the questionnaire administered, 3,728 copies (representing 76% of the total copies) were successfully retrieved and only 3,214 copies (about 65%) were duly completed and found useful for data analysis. The Statistical Product and Service Solutions (SPSS v 20.0) was employed for data analysis. The statistical tools used for data analysis were mean to answer research questions and standard deviation to validate the closeness of the respondents from the mean and from each other in their responses while a two-tailed t-test was used to test whether male and female farmers differed in their response, using a 0.05 level of significance. Decisions were made at mean criterion value point of 2.50. To arrive at 2.50 criterion value, the average of the mean weights was calculated. Items with mean values equal or greater than 2.50 were regarded as "Adverse Effect (AE)" while with mean values lower the value point were regarded as "Non Adverse Effect (NAE)" affecting the farming profession and farmer and the farming families in the Niger Delta region.

### RESULTS

Data on Table 1 revealed that seventeen items (S/N. 2 and 6-21) as responded to by farmers were adverse effects (AE) of altered climate on farming profession in the study area as their means ranged from 2.52 to 3.39 which is higher than the criterion mean value of 2.50. The remaining four items (S/N 1, 3-5) were regarded as non adverse effects (NAE) on the farming profession in the region as their means were 1.17, 2.38, 2.40, and 2.41 all of which were lower than 2.50 criterion mean. The table also showed that the standard deviation (SD) of the 21 items ranged from 0.11 to 1.19; indicating that the respondents were close to the mean and to one another in their responses. Data on Table 1 revealed that there is no significant difference (S) in the opinions of male and female farmers in seven items (S/N 1-3, 5, 9, 13, and 15), and a non-significant (NS) difference in fourteen items (S/N. 4, 6-8, 14, 16-21). However, the cluster value indicated that the difference in their opinions is NS. The null hypothesis of no significant difference of the items with remark NS was upheld as  $p$ -value is greater than the  $\alpha$ -value ( $p \geq \alpha$ ) at the t-calculated value of the items. At the cluster value,  $p=0.10$  and  $t=0.56$ , while significant level  $=0.05$ . Thus, the null hypothesis (H<sub>01</sub>) of no significant difference is not rejected, but upheld as  $p \geq \alpha$  ( $0.10 > 0.05$ ).

In Table 2, data indicate that eleven items (S/N 2, 3, 5,

**Table 1.** Mean, standard deviation and t-test scores of respondents on the effects of altered climate on the farming profession in the Niger Delta region (N=3,214).

S/N	Effects on the farming profession	$\bar{X}$	SD	R	H <sub>01</sub>		
					t	*Sig	R
1	Reduced water availability for irrigation	1.17	1.09	NAE	2.25	0.03	S
2	Damage of stored grain	2.52	1.11	AE	-3.92	0.00	S
3	Spread of plant and animal diseases	2.38	0.97	NAE	-5.73	0.00	S
4	Invasion of new plant and animal pests	2.40	1.19	NAE	1.34	0.05	NS
5	Invasion of new plant and/or animal diseases	2.41	0.97	NAE	6.09	0.00	S
6	Rapid spread of weeds and pest in the farm	2.55	1.00	AE	-8.14	0.08	NS
7	Increased difficulty level and cost in the control of weed and pest	2.87	1.12	AE	6.16	0.15	NS
8	Change in rainfall pattern affecting historical patterns of cultivation	2.54	1.08	AE	9.20	0.15	NS
9	Poorer germination and birth rate	3.26	0.71	AE	0.13	0.03	S
10	Diminished plant and animal yield	2.84	0.88	AE	1.01	0.20	NS
11	Reducing marketability of produce	3.38	0.72	AE	-1.07	0.18	NS
12	Stunting of animal and plant growth	3.00	0.71	AE	2.56	0.09	NS
13	Increasing death scorching rate in production	2.62	0.72	AE	-0.15	0.01	S
14	Flooding of farmland and animal houses	3.95	0.11	AE	0.51	0.16	NS
15	Altering nature of work done on the farm	2.75	0.97	AE	-6.92	0.03	S
16	Increasing difficulty of work done on the farm	2.84	0.88	AE	1.19	0.15	NS
17	Increased labour cost due work difficulty	3.38	0.72	AE	0.86	0.09	NS
18	Increasing labour hours on the farm due to altered and increased difficulty of work in the farm	3.00	0.71	AE	3.67	0.19	NS
19	Cost of production (input and labour cost)	3.39	0.76	AE	1.95	0.17	NS
20	Declining net profit from farming	2.94	0.92	AE	1.79	0.16	NS
21	Lowered farming business expansion opportunities due higher cost of production	2.53	0.55	AE	-0.98	0.08	NS
Cluster value		2.80	0.85	AE	0.56	0.10	NS

R: Remark; AE; adverse effect; NAE: non adverse effect; NS: non significant; S: significant.  $p = *Sig$  (2-tailed).

8, 9, and 11-16) had mean values within 2.53 and 3.95. These mean values are higher than the 2.50 criterion mean values; indicating that altered climate by the items adversely affect (AE) the farmer and the farming families in the Niger Delta region of Nigeria. The other five items (S/N 1, 4, 6, 7, and 10) had NAE on the farmer and the farming families in the region as their means were lower than 2.50 criterion mean. The table also showed that the SD of the 16 items ranged from 0.62 to

1.20; indicating that the respondents were close to the mean and to one another in their responses. Data in Table 2 revealed that there is no significant difference (S) in the opinions of male and female farmers in eight items (S/N 1-4, 6-8 and 10) and a NS difference in eight items (S/N. 5, 9, 11-16). However, the cluster value indicated that the difference in their opinions is NS. The null hypothesis of no significant difference of the items with remark NS was upheld as  $p$ -value is greater

than the  $\alpha$ -value ( $p \geq \alpha$ ) at the t-calculated value of the items. At the cluster value,  $p=0.10$  and  $t=0.98$ , while significant level=0.05. Thus, the null hypothesis (H<sub>02</sub>) of no significant difference is not rejected, but upheld as  $p \geq \alpha$  ( $0.10 > 0.05$ ).

## DISCUSSION

Niger Delta like most coastal low lying regions of

**Table 2.** Mean, standard deviation, and t-test scores of Respondents on the effects of the altered climate on the living conditions of the farmer and the farming families in the Niger Delta region of Nigeria (N=3,214).

S/N	Effects on the livelihood of the farmer and the farming families	$\bar{X}$	SD	Dec	H <sub>02</sub>		
					t	*Sig	R
1	Drying up of ponds and streams	1.19	1.15	NAE	3.75	0.04	S
2	Pollution of clean water sources	3.42	0.89	AE	6.41	0.02	S
3	Risk of sickness	2.53	1.11	AE	-3.92	0.01	S
4	Spread of disease	2.38	0.97	NAE	-5.73	0.04	S
5	Damage to buildings	2.70	1.20	AE	1.34	0.20	NS
6	Damage to roads and bridges leading to homes	2.41	0.97	NAE	6.09	0.04	S
7	Roof damage by acid rain	2.15	1.00	NAE	-8.14	0.04	S
8	Drainage system damage	2.87	1.12	AE	6.16	0.02	S
9	Poverty level	3.51	1.15	AE	0.82	0.11	NS
10	Loss of live (mortality rate)	2.41	0.95	NAE	2.70	0.02	S
11	Hotter environment leading to heat stress	2.58	0.99	AE	4.59	0.17	NS
12	Flooding of houses and neighborhood	3.95	0.11	AE	0.51	0.16	NS
13	Displacement of farmers from their homes	3.51	0.62	AE	-0.93	0.20	NS
14	Food shortage due to poor produce	2.64	0.95	AE	0.59	0.16	NS
15	Increasing cost of living	2.89	1.06	AE	0.65	0.17	NS
16	Increasing loss of (inherited) lands due to high cost of land reclaiming	3.21	1.12	AE	0.81	0.18	NS
Cluster value		2.77	0.96	AE	0.98	0.10	NS

R: Remark; AE; adverse effect; NAE: non adverse effect; NS: non-significant; S: significant. p = \*Sig (2-tailed).

the world is constantly faced with flooding of various degrees.

However, due to increased and varying extent of precipitation attributable to climate change, the occurrence of flooding has increased with rivers and oceans easily overflowing their banks. This was observed in the 2012 flooding that impacted negatively on agriculture in the region. Altered climate have resulted to more adverse effects affecting cultivation and general farming in the region. In Table 1, items such as “reducing marketability of produce”, “increased labour cost due work difficulty”, and “cost of production (input and labour cost)” had high mean values of 3.38,

3.38, and 3.39 respectively. Indications like this reveal that farmers could be spending more than usual. Reduced marketability means reduced sales of products or value of farm produces, while increased labour and other input costs results to increased cost of production. The scenario will definitely affect the economic viability of farming as a sustainable means of livelihood. The findings of this study as presented in Table 1 are in agreement with that of Uyigue and Agho (2007), Bhusal (2009), Miguel and Koohafkan (2010), and Ikehi et al. (2014) that stated that difficulty and cost of agricultural production will increase with decreasing returns to the farmer. The authors

anticipated more problems (which are now occurring) in farming resulting from altered climate.

Increasing adverse on farming profession tends to directly impact on the livelihood of the farmer and the farming families. While most projected effects holds true, however, increase in mortality rate tied directly to altered climate in the region was not indicated by the respondents. This finding disagrees with Cruz et al. (2007) who stated that there will be increased death rate due to factors favoured by altered climate. Also, drying up of ponds and other sources of water in the region as projected does not hold true for the region as the

area is coastal and with abundance of water.

This view is supported by Uyigue and Agho (2007) that stated that increased precipitation will cause excess of water, even flooding and not drought. As stated, wet regions will receive more rain while dry regions will become even drier. However, the adverse effects of altered climate is bearing had on the farmer and his farming families as indicated by the response of the farmers in Table 2. In Table 2, "pollution of clean water sources", "poverty level", "flooding of houses and neighborhood", and "displacement of farmers from their homes" had high mean values. The respondents are not far from reality. Overflowing of rivers and heavy runs offs could lead to redirection of polluted or mud water into clean streams used by rural farmers as source of clean water. With decreased sales and increased cost of production, returns tend to reduce leading to rising poverty level as reported by the farmers. Risen water level means flooding and destruction of routes and residences leading to farmers' displacement. These findings are not at divergent with that of authors such as Boko et al. (2007), IPCC (2007), Wolfe (2007), Apata (2010), and Ikehi and Zimoghen (2015) who outlined the effects of altered climate on humans as mainly negative affecting livelihood and families in the world, especially in African countries.

Findings of the study revealed that significant difference does not exist between the mean responses of the male and female farmers on altered climate and responsible livelihood of farming families in Niger Delta region of Nigeria, as indicated by t-test for both hypotheses. Therefore, any observed difference is not a statistical difference, but a mere chance which could have resulted from sampling error.

## CONCLUSIONS AND RECOMMENDATIONS

Findings of this study served as a premise for making the following conclusions: altered climate has become a threat to sustainability of farming as a profession in the Niger Delta region of Nigeria; flooding is a major threat to sustainable agriculture and livelihood in the region; poverty level is on the rise as a result of low net profit from agriculture caused by the adverse effects of altered climate in the region.

In recommendation, since most crop farmers in the region practice rain-fed agriculture, it is necessary for the government and other relevant authorities to constantly provide weather information such as rainfall distribution ahead of time to help the farmers plan. This could be done through the radio or through the extension agents. While government and organized interventions are necessary, the farmers could construct dykes and barriers as well as gutters around their building to redirect flood away from their homes and farmlands. With rising poverty level among the already indigent farmers, farm input purchase capacity of the farmers will be affected,

directly affecting production. Farmers in the region can be encouraged by providing incentives and subsidizing inputs for them by federal and state government as well as other non-governmental organizations. This will go a long way in improving production and discourage migration when profit from farming profession continue to reduce or standard of living worsens due to altered climate in the region.

## Conflict of interests

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

## Economic viability of implementing a diesel generator group in a grain storage facility located in the City of Cascavel, State of Paraná, Brazil

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The objective of the present study is to verify the viability of implementing a diesel-generating group, which would work at peak times, in a grain-processing facility located in the town of Cascavel, in western region of Paraná, Brazil. The demand and consumption of electrical energy for the unit were determined for four operational scenarios. For each of these scenarios, the annual cost of using a diesel-fueled generator was compared to the cost of using electrical energy under three pricing systems. The criteria used for making investment decisions were the Internal Rate of Return and the Payback Discount method. It was determined the minimum annual use necessary for the implementation of a diesel generator to become viable. In the most critical scenario of the operation, the implementation of a generator becomes viable in the fourth year of use. The main results allowed concluding that implementing a diesel generator group at peak times is only not viable when using a conventional rate system and when demand and consumption are low.

**Key words:** Storage, peak time, electrical energy production.

### INTRODUCTION

Brazilian agriculture has gained international attention due to its competitive potential and its agriculture and livestock expansion possibilities. In the last survey on grain production, CONAB (Companhia Nacional de Abastecimento / National Food Supply Company) verified that the 2012/2013 crop reached 187.09 million tons, 12.6% more than the 2011/2012 crop (CONAB, 2013).

The harvest flow system involves the process of storing agricultural products, an extremely important step for maintaining grain quality, since it is useless to produce well if this production could be compromised by an inadequate storage process (Alencar et al., 2009; Kolling et al., 2012).

A storage unit consists of a system that has been

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appropriately designed and structured to receive, clean, dry, store, and expedite grains. To accomplish this, the unit must be comprised of efficient structures, processing equipment, and transportation equipment (Konopatzki et al., 2006). These units are large complexes that consume high quantities of energy, mainly because of the number of electric motors they use (Teixeira et al., 2007; Costa et al., 2010). When it is used a lot of energy in the production process, optimum use of energy becomes very vital for agricultural productions (Mousavi-Avval et al., 2010; Ommani, 2011). Consequently, electrical energy consumption becomes a significant monthly utility expense for these facilities.

In Brazil, the rates charged for supplying electricity are linked to the characteristics of each consuming unit and categorized under three modalities, which vary according to the consumer's power demands and voltage supplied. The first modality, referred to as Conventional Rate (Flat Rate), consists of a single price for power demands (US\$/kWh) and for the consumption of energy (US\$/kWh). The other two modalities are variable and based on seasonal time (in Brazil, they are called "green" or "blue" rates, described below). These differ from the conventional rate because they are based on Time of Use (TOU), that is, they are higher at peak times (the peak time corresponds to the three consecutive weekday hours, normally between 6 and 9 PM, in which the supplier's rate increases significantly, because there is an increase in consumption during these times at a national level, overloading the energy generation, transmission, and distribution systems). The "green" rate consists of a single price for power and two prices for energy consumption (one for peak times and one for off-peak times). The "blue" rate, however, consists of two prices for power and two prices for energy consumption (peak and off-peak times for both cases).

At harvest time, one storage unit may function without interruptions, even during peak times, increasing electrical energy expenses by four hundred percent during this period. To minimize costs with electricity during peak hours, one option is to make use of alternative energy sources, such as, for example, diesel generator groups (Petrilli et al., 2013). Mbodji et al. (2013) showed the importance of decentralized application of multi-sources electrical systems. Such a management allows optimizing the system in function of the costs.

A diesel generator group is understood as the combination of a diesel-fueled motor and an alternating current generator, with supervision and control components that allow it to function autonomously. Its purpose is to supply electrical energy generated by diesel oil. According to Masseroni and Oliveira (2012), the use of this type of equipment at peak hours can represent a 30% savings on the monthly energy bill. However, the decision to implement a generator group should only be made once a detailed study of its economic viability has

been executed.

This project studied the economical implementation of a diesel generator group at peak hours in a grain processing/storage unit situated in the town of Cascavel, in the western region of Paraná - Brazil.

## MATERIALS AND METHODS

The present study was executed in a grain processing/storage unit situated in the town of Cascavel, in the western region of Paraná - Brazil. The static storage capacity for this particular unit is 30 thousand tons, equally distributed among 8 metallic silos. During the harvest period, it processes corn and soy crops. The electric motors involved in grain processing in this unit are listed in Table 1.

To estimate the local demand for electricity, four operational scenarios were considered, established according to the characteristics of the product the unit receives.

Scenario 1 - clean and dry product with this configuration, the product does not go through any processing stages; it is simply transported to storage. In this scenario, energy consumption occurs from using the equipment 1, 10, 11, 12, and 13 (listed in Table 1) and the demand for electricity is 38.7 kVA. All these equipment are used simultaneously;

Scenario 2 - clean and damp product: With this configuration, the product must go through a drying process before it can be stored. As such, energy consumption occurs from using the equipment listed under scenario 1 plus items 5 to 9 (from Table 1). In this process, the demand for electricity is 101.4 kVA. All these equipment are used simultaneously.

Scenario 3 - dirty and dry product: With this configuration, the product must be cleaned prior to storage. As such, energy consumption occurs from using equipment 1 to 4 and 10 to 13 (from Table 1). In this process, the demand for electricity is 48.2 kVA. All these equipment are used simultaneously.

Scenario 4 - dirty and damp product: This is the most critical operational configuration because all processing stages must be completed before storage. As such, all of the equipment listed in Table 1 is used and the demand for energy equals 110.9 kVA. All these equipment are used simultaneously.

One generator group was attributed to each scenario described. This attribution was performed using *Cummins Power* generators and a safety margin of 20% over the scenario's demand. The generators selected for each case are listed in Table 2. The generator group will be used to supplement the demand for electrical energy only at peak times (6 to 9 PM), when the supplier increases the rates charged.

For each scenario, the viability of implementing the generator group at peak times was verified, comparing the annual costs of using the diesel generator with the annual costs of using electrical energy. The costs of using diesel-generated energy were calculated according to the information listed in Table 3.

Three rate systems were used to calculate the costs incurred from using electrical energy: 1-Conventional rate application; 2-"Blue" TOU rate application; and 3-"Green" TOU rate application. The rates used in this study (Table 4) refer to the consumption group supplied between 30 and 44 kV, and have been calculated according to the Companhia Paranaense de Energia (COPEL, 2013).

The costs incurred with the diesel generator group, as well as the costs incurred with electrical energy, were calculated for one year according to total peak periods (60 h per month or 720 h per year). Under these conditions, the equipment's operational life is 14 years.

To complete an economic analysis, the Internal Rate of Return (IRR) and the Payback Discount (PBD) method were used (Casarotto and Kopitke, 2010). The Minimum Acceptable Rate of

**Table 1.** Electric motors used in grain processing.

Item	Equipment identification	Place of installation	Power (cv)	Efficiency (%)	Power factor	Demand (kVA)
1	Hopper lifter	Reception	25	91.0	0.83	14.06
2	MPL fan	Cleaning Machine	7.5	88.0	0.82	4.42
3	MPL sieve motor	Cleaning Machine	7.5	88.0	0.82	4.42
4	MPL residue motor	Cleaning Machine	1	79.5	0.82	0.65
5	Dryer lifter	Grain Dryer	20	90.2	0.83	11.35
6	Dryer fan	Grain Dryer	30	91.0	0.84	16.68
7	Dryer fan	Grain Dryer	30	91.0	0.84	16.68
8	Dryer fan	Grain Dryer	30	91.0	0.84	16.68
9	Dryer motor	Grain Dryer	2	82.5	0.78	1.32
10	Screw conveyor	Storage	7.5	88.0	0.82	4.42
11	Conveyor belt	Storage	7.5	88.0	0.82	4.42
12	Conveyor belt	Storage	20	90.2	0.83	11.35
13	Lifter	Storage	7.5	88.0	0.82	4.42

**Table 2.** Characteristics of the generators attributed.

Scenario	Generator model	Nominal power (Use at peak - kVA)	Consumption (liters/hour)			
			Complete	$\frac{3}{4}$	$\frac{1}{2}$	$\frac{1}{4}$
1	C40D6	48	11	9	7	5
2	C110D6	128	29	22	16	9
3	C50D6	56	14	12	9	6
4	C110D6	128	29	22	16	9

**Table 3.** Diesel-generated energy costs.

	Cost
48 kVA generator purchase	US\$ 19,772.73
56 kVA generator purchase	US\$ 20,636.36
128 kVA generator purchase	US\$ 26,727.27
Equipment maintenance	2% of the cost of the equipment <sup>1</sup>
Installation	3% of the cost of the equipment <sup>1</sup>
Lubricating oil	US\$ 5.454/liter <sup>2</sup>
Cost of fuel	US\$ 0.964/liter

<sup>1</sup>Information from the Manufacturer. <sup>2</sup>Lubricating oil consumption calculated at 10 L for 100 hours of use.

**Table 4.** Fees charged to the 3 rate modalities during peak times.

Rate	Peak time cost	
	Demand (US\$/kW)	Energy (US\$/kWh)
"Blue" TOU	12.2955	0.1609
"Green" TOU	3.7500	0.4567
Conventional	12.6091	0.1074

Return (MARR) considered for this study was 8% per year. Additionally, through a series of graphs, it was possible to determine the

minimum annual use, in hours, necessary for the implementation of a diesel generator group to become viable economically.

Table 5. Cash flow scenario 1.

Year	"Blue" TOU			"Green" TOU			Conventional		
	CF(US\$)	PV (US\$)	CPV (US\$)	CF (US\$)	PV (US\$)	CPV (US\$)	CF (US\$)	PV (US\$)	CPV (US\$)
0	-20,365.91	-20,365.91	-20,365.91	-20,365.91	-20,365.91	-20,365.91	-20,365.91	-20,365.91	-20,365.91
1	3,421.23	3,167.81	-17,198.10	7,838.98	7,258.31	-13,107.60	2,033.86	1,883.20	-18,482.71
2	3,421.23	2,933.15	-14,264.95	7,838.98	6,720.66	-6,386.94	2,033.86	1,743.70	-16,739.01
3	3,421.23	2,715.88	-11,549.07	7,838.98	6,222.83	-164.11	2,033.86	1,614.54	-15,124.47
4	3,421.23	2,514.71	-9,034.36	7,838.98	5,761.88	5,597.77	2,033.86	1,494.94	-13,629.52
5	3,421.23	2,328.43	-6,705.93	7,838.98	5,335.07	10,932.85	2,033.86	1,384.21	-12,245.32
6	3,421.23	2,155.95	-4,549.98	7,838.98	4,939.88	15,872.73	2,033.86	1,281.67	-10,963.64
7	3,421.23	1,996.25	-2,553.72	7,838.98	4,573.97	20,446.70	2,033.86	1,186.73	-9,776.91
8	3,421.23	1,848.38	-705.34	7,838.98	4,235.15	24,681.85	2,033.86	1,098.83	-8,678.08
9	3,421.23	1,711.47	1,006.13	7,838.98	3,921.44	28,603.29	2,033.86	1,017.43	-7,660.64
10	3,421.23	1,584.69	2,590.82	7,838.98	3,630.96	32,234.25	2,033.86	942.07	-6,718.58
11	3,421.23	1,467.31	4,058.13	7,838.98	3,362.00	35,596.25	2,033.86	872.29	-5,846.29
12	3,421.23	1,358.62	5,416.74	7,838.98	3,112.96	38,709.22	2,033.86	807.67	-5,038.62
13	3,421.23	1,257.98	6,674.72	7,838.98	2,882.37	41,591.59	2,033.86	747.84	-4,290.77
14	3,421.23	1,164.80	7,839.52	7,838.98	2,668.87	44,260.46	2,033.86	692.45	-3,598.33

CF = Cash flow; PV = present value; CPV = cumulative present value.

## RESULTS AND DISCUSSION

### Payback Discount (PBD) method

For the first scenario, in which there is a lower demand for electricity (38.7 kVA), the cash flow obtained is presented in Table 5. In the cash flow, the 1st column, at year 0, indicates the cost of purchasing and installing the diesel generator. The remaining values, from years 1 to 14, indicate the avoidable electrical energy costs paid to a supplier, minus the costs of operating and maintaining the diesel generator. When comparing generator group usage with electrical energy usage under the "blue" TOU rate system, the rate of return on invested capital is 9 years. When considering the "green" TOU rate system, the rate of return is 4 years. When the conventional rate is used, the rate charged at peak times is the lowest among the three modalities, and the implementation of a generator group is not viable economically.

In the second scenario, where the demand for electrical energy is 101.4 kVA, the use of a generator group of larger capacity and higher cost was considered in the calculations. Table 6 presents the cash flow for this configuration.

In Table 6, it is possible to observe that for the "blue" TOU rate system, the equipment's implementation becomes viable at year 4. When the "green" TOU rate is used, the rate of return on invested capital is as early as 2 years. This result is very interesting to an investor, since the rate of return is considerably low. However, when compared to the conventional rate, the implementation of this equipment is only viable in year 7.

For the third scenario, where the demand for electrical

energy is 48 kVA, the cash flow obtained (Table 7) is very similar to the one generated in the first scenario, since the energy demand and generator costs are similar.

As in the first scenario, the "blue" TOU system, the rate of return on capital invested is 9 years. However, when using the "green" TOU, the rate of return is only 3 years. In contrast, when using the conventional rate, the equipment's implementation is not viable economically.

Table 8 presents the cash flow obtained for the fourth scenario, where the demand for electrical energy is 110.9 kVA. When it is used the "blue" TOU rate system or the conventional system, the generator's implementation becomes economically viable at year 4. When it is used the "green" TOU, the rate of return is 2 years.

### Results obtained using the Internal Rate of Return method (IRR)

The IRR is based on the same cash flow values previously presented. The results for each rate system in the four scenarios are listed in Table 9.

For scenario 1, the investment should only occur as a replacement for the "blue" or "green" TOUs, because the IRR is higher than the Minimum Acceptable Rate of Return- MARR (8% per year). Similarly, when conventional rates are used, it is best not to implement the generator. Under scenario 2, the investment can be made under any circumstance, since the IRR is superior to the MARR. In fact, the IRR value for the "green" TOU rate is well above the MARR, indicating that this configuration is the most interesting when considering the implementation of a diesel-fueled generator. Under the

**Table 6.** Cash Flow Scenario 2.

Year	"Blue" TOU			"Green" TOU			Conventional		
	CF (US\$)	PV (US\$)	CPV (US\$)	CF (US\$)	PV (US\$)	CPV (US\$)	CF (US\$)	PV (US\$)	CPV (US\$)
0	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09
1	9,230.96	8,547.19	-18,981.91	20,827.54	19,284.76	-8,244.33	5,589.10	5,175.09	-22,354.00
2	9,230.96	7,914.06	-11,067.85	20,827.54	17,856.26	9,611.93	5,589.10	4,791.75	-17,562.24
3	9,230.96	7,327.83	-3,740.01	20,827.54	16,533.57	26,145.50	5,589.10	4,436.81	-13,125.43
4	9,230.96	6,785.03	3,045.02	20,827.54	15,308.87	41,454.37	5,589.10	4,108.16	-9,017.28
5	9,230.96	6,282.44	9,327.46	20,827.54	14,174.88	55,629.25	5,589.10	3,803.85	-5,213.43
6	9,230.96	5,817.07	15,144.53	20,827.54	13,124.88	68,754.13	5,589.10	3,522.08	-1,691.34
7	9,230.96	5,386.18	20,530.70	20,827.54	12,152.67	80,906.80	5,589.10	3,261.19	1,569.84
8	9,230.96	4,987.20	25,517.90	20,827.54	11,252.47	92,159.27	5,589.10	3,019.62	4,589.46
9	9,230.96	4,617.78	30,135.68	20,827.54	10,418.96	102,578.23	5,589.10	2,795.94	7,385.40
10	9,230.96	4,275.72	34,411.40	20,827.54	9,647.18	112,225.41	5,589.10	2,588.84	9,974.24
11	9,230.96	3,959.00	38,370.40	20,827.54	8,932.58	121,157.99	5,589.10	2,397.07	12,371.31
12	9,230.96	3,665.74	42,036.15	20,827.54	8,270.90	129,428.89	5,589.10	2,219.51	14,590.82
13	9,230.96	3,394.20	45,430.35	20,827.54	7,658.24	137,087.13	5,589.10	2,055.10	16,645.92
14	9,230.96	3,142.78	48,573.13	20,827.54	7,090.97	144,178.10	5,589.10	1,902.87	18,548.79

**Table 7.** Cash Flow Scenario 3.

Year	"Blue" TOU			"Green" TOU			Conventional		
	CF (US\$)	PV (US\$)	CPV (US\$)	CF (US\$)	PV (US\$)	CPV (US\$)	CF (US\$)	PV (US\$)	CPV (US\$)
0	-21,255.45	-21,255.45	-21,255.45	-21,255.45	-21,255.45	-21,255.45	-21,255.45	-21,255.45	-21,255.45
1	3,678.74	3,406.24	-17,849.22	9,200.92	8,519.37	-12,736.09	1,944.52	1,800.48	-19,454.97
2	3,678.74	3,153.92	-14,695.29	9,200.92	7,888.30	-4,847.78	1,944.52	1,667.11	-17,787.86
3	3,678.74	2,920.30	-11,774.99	9,200.92	7,303.99	2,456.21	1,944.52	1,543.62	-16,244.24
4	3,678.74	2,703.98	-9,071.01	9,200.92	6,762.95	9,219.15	1,944.52	1,429.28	-14,814.96
5	3,678.74	2,503.69	-6,567.33	9,200.92	6,261.99	15,481.15	1,944.52	1,323.41	-13,491.56
6	3,678.74	2,318.23	-4,249.10	9,200.92	5,798.14	21,279.28	1,944.52	1,225.38	-12,266.18
7	3,678.74	2,146.51	-2,102.59	9,200.92	5,368.65	26,647.93	1,944.52	1,134.61	-11,131.57
8	3,678.74	1,987.51	-115.08	9,200.92	4,970.97	31,618.90	1,944.52	1,050.56	-10,081.01
9	3,678.74	1,840.28	1,725.20	9,200.92	4,602.75	36,221.65	1,944.52	972.74	-9,108.26
10	3,678.74	1,703.97	3,429.17	9,200.92	4,261.81	40,483.46	1,944.52	900.69	-8,207.58
11	3,678.74	1,577.75	5,006.92	9,200.92	3,946.12	44,429.57	1,944.52	833.97	-7,373.61
12	3,678.74	1,460.88	6,467.79	9,200.92	3,653.81	48,083.39	1,944.52	772.20	-6,601.41
13	3,678.74	1,352.66	7,820.46	9,200.92	3,383.16	51,466.54	1,944.52	715.00	-5,886.42
14	3,678.74	1,252.47	9,072.92	9,200.92	3,132.55	54,599.10	1,944.52	662.03	-5,224.38

third scenario, the values are a close match to those in the first scenario. As such, the investment should only be made in replacement to the "blue" or "green" TOU rate systems. For the fourth scenario, all of the values are favorable to the implementation project.

#### **Graphs comparing the costs of using electrical energy or using a generator group**

The graph in Figure 1 presents a comparison between

yearly electrical energy usage and generator group costs for scenario 1. On Figure 1, it is possible to see that for annual usage above 250 h, the implementation of a diesel generator group is economically viable in comparison to the cost of electrical energy charged at the "green" TOU rate system. For the "blue" TOU rate system, the investment is only viable at 540 usage hours. Moreover, when compared to the conventional rate, the implementation of a generator group is not viable economically.

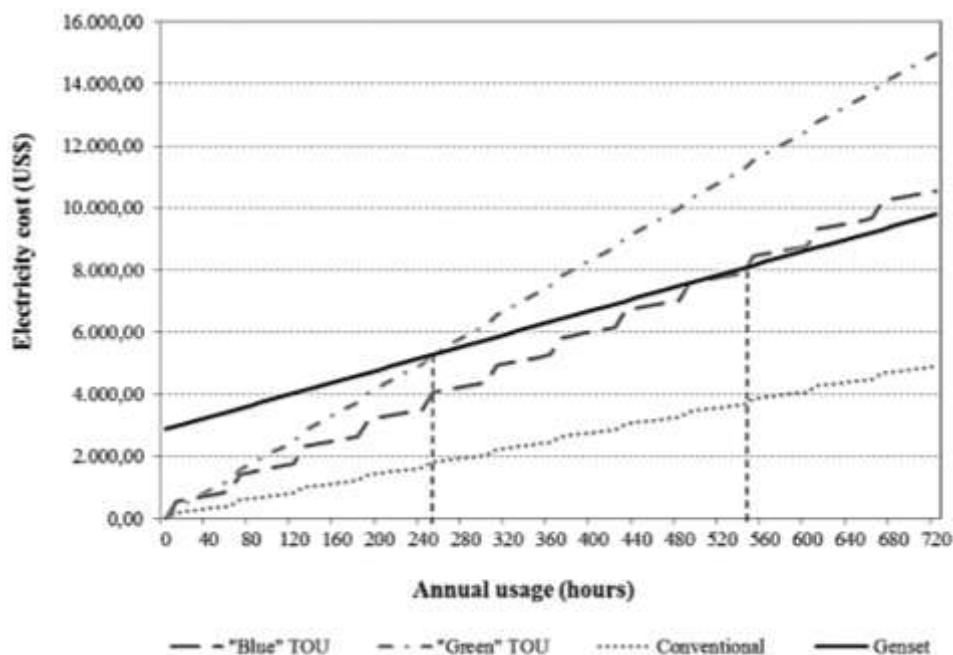
Figure 2 presents a cost comparison for scenario 2.

**Table 8.** Cash flow scenario 4.

Year	"Blue" TOU			"Green" TOU			Conventional		
	CF(US\$)	PV(US\$)	CPV(US\$)	CF(US\$)	PV(US\$)	CPV(US\$)	CF(US\$)	PV(US\$)	CPV(US\$)
0	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09	-27,529.09
1	8,761.32	8,112.33	-19,416.76	25,883.86	23,966.53	-3,562.56	9,194.14	8,513.09	-19,016.00
2	8,761.32	7,511.42	-11,905.34	25,883.86	22,191.23	18,628.68	9,194.14	7,882.49	-11,133.51
3	8,761.32	6,955.02	-4,950.33	25,883.86	20,547.44	39,176.12	9,194.14	7,298.60	-3,834.91
4	8,761.32	6,439.83	1,489.50	25,883.86	19,025.41	58,201.52	9,194.14	6,757.96	2,923.05
5	8,761.32	5,962.81	7,452.31	25,883.86	17,616.12	75,817.64	9,194.14	6,257.37	9,180.43
6	8,761.32	5,521.12	12,973.43	25,883.86	16,311.22	92,128.86	9,194.14	5,793.87	14,974.29
7	8,761.32	5,112.14	18,085.57	25,883.86	15,102.98	107,231.84	9,194.14	5,364.69	20,338.98
8	8,761.32	4,733.47	22,819.04	25,883.86	13,984.24	121,216.08	9,194.14	4,967.31	25,306.29
9	8,761.32	4,382.84	27,201.88	25,883.86	12,948.37	134,164.46	9,194.14	4,599.36	29,905.65
10	8,761.32	4,058.19	31,260.06	25,883.86	11,989.23	146,153.69	9,194.14	4,258.66	34,164.31
11	8,761.32	3,757.58	35,017.64	25,883.86	11,101.14	157,254.83	9,194.14	3,943.21	38,107.52
12	8,761.32	3,479.24	38,496.88	25,883.86	10,278.84	167,533.67	9,194.14	3,651.12	41,758.63
13	8,761.32	3,221.52	41,718.40	25,883.86	9,517.44	177,051.11	9,194.14	3,380.66	45,139.30
14	8,761.32	2,982.89	44,701.29	25,883.86	8,812.44	185,863.55	9,194.14	3,130.25	48,269.54

**Table 9.** Internal rate of return for each rate system and scenario studied.

Scenario	"Blue" TOU	"Green" TOU	Conventional
	IRR (%)	IRR (%)	IRR (%)
1	14	38	5
2	33	76	18
3	15	43	3
4	31	94	33



**Figure 1.** Costs incurred with electrical energy and generator group for scenario 1.

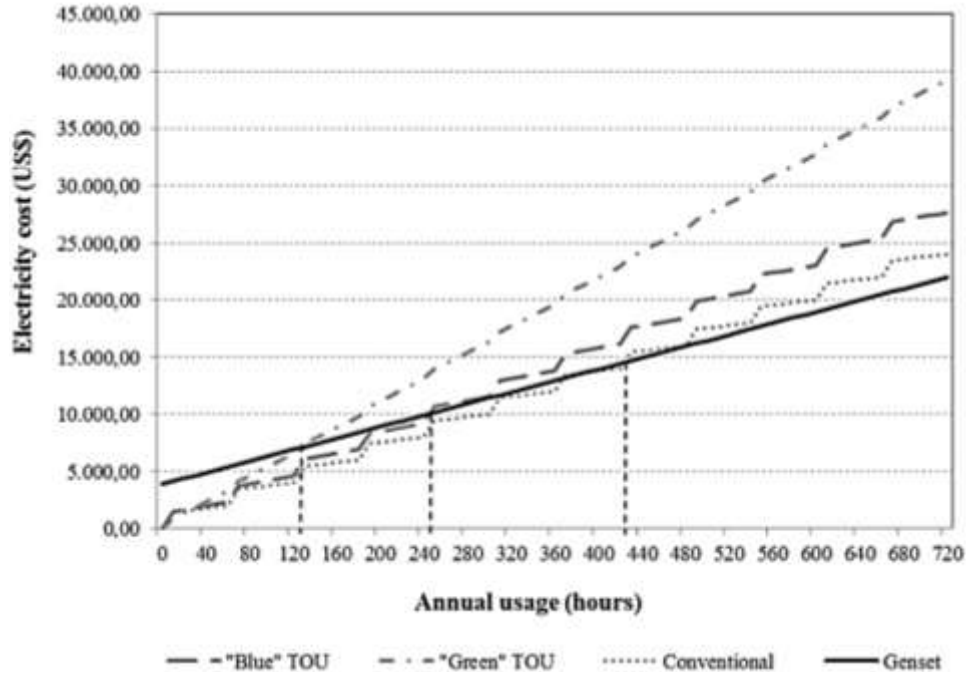


Figure 2. Costs incurred with electrical energy and generator group for scenario 2.

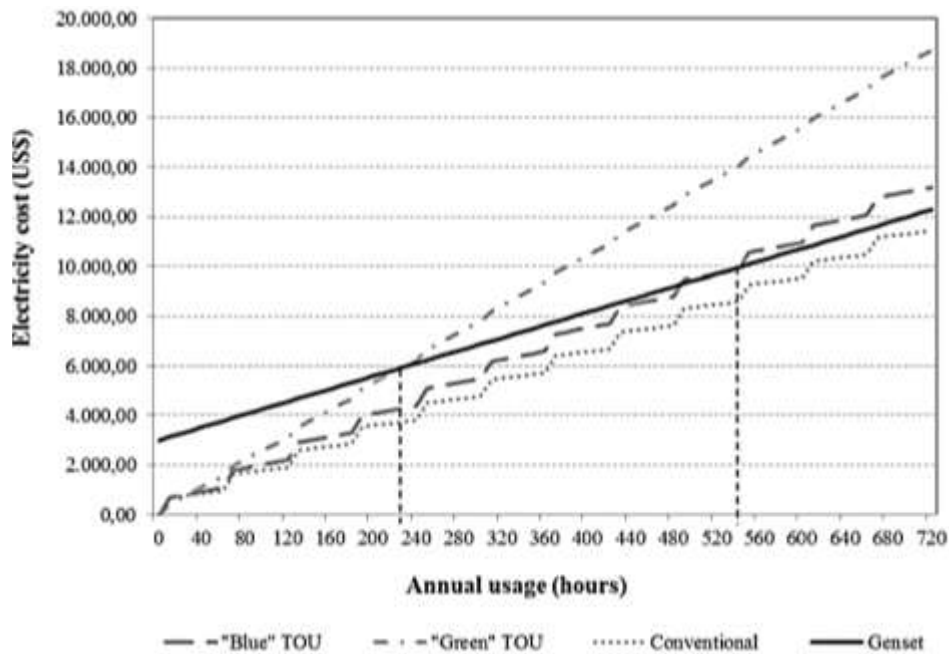


Figure 3. Costs incurred with electrical energy and generator group for scenario 3.

Figure 2 shows that 130 annual usage hours are needed for the implementation of the generator, to become viable under the "green" TOU rate system. Under the "blue" TOU rate system, 250 usage hours are needed, and for the conventional system, 430 h.

Figure 3 represents a cost comparison for scenario 3. The graph in Figure 3 shows that for the "green" TOU rate, the generator's implementation is viable after 230 usage hours. Under the "blue" TOU rate system, at least 540 usage hours are needed, and in comparison to the

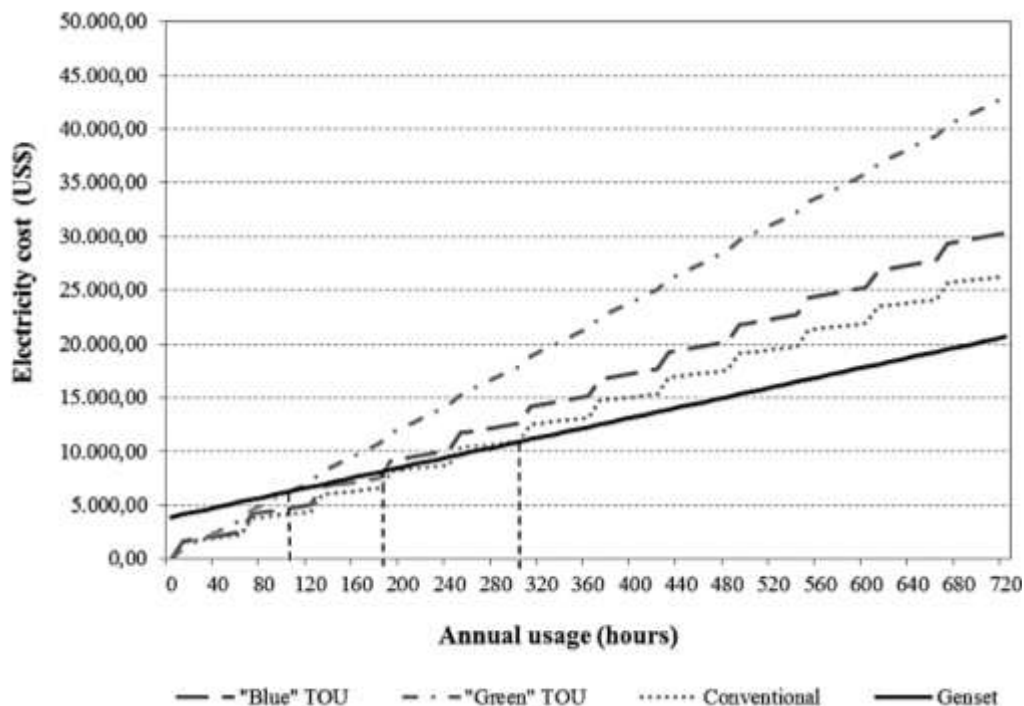


Figure 4. Costs incurred with electrical energy and generator group for scenario 4.

conventional system, the generator's implementation is not viable economically.

Figure 4 represents a cost comparison for scenario 4. Figure 4 shows that under the "green" TOU rate system, investment becomes viable after 100 hours of use. Comparatively, under the "blue" TOU system, it becomes viable to implement the generator after 180 annual usage hours, and under the conventional system this number rises to 300 h.

## Conclusions

According to the data obtained from the assessments performed in this article, it is possible to conclude that:

1. The use of a diesel generator group can reduce costs during the times of day in which the electrical energy rate is highest (peak times);
2. Substituting the "green" TOU rate for a generator group is an interesting option, since the Payback Discount is very low;
3. For the "blue" TOU rate system, the implementation of a generator group presented positive economic results. However, it is important to also consider the balance between costs and number of usage hours of the generator;
4. Implementing a diesel generator group at peak times is only not viable when using a conventional rate system and when demand is low.

## Conflict of interest

The authors have not declared any conflict of interest.

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## Short Communication

**Auxin induced rooting of cactus pear (*Opuntia ficus-indica* L. Miller) cladodes for rapid on-farm propagation**Usman Inuwa Shehu<sup>1\*</sup>, Lawan Abdu Sani<sup>2</sup> and Abdulrazak Baba Ibrahim<sup>3</sup><sup>1</sup>Department of Plant Science, Ahmadu Bello University, Zaria, Nigeria.<sup>2</sup>Department of Plant Biology, Bayero University, Kano, Nigeria.<sup>3</sup>Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

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The effect of exogenous auxins on the development of root system in cladodes of *Opuntia ficus-indica* was tested using different concentrations of indole butyric acid (IBA), naphthalene acetic acid (NAA) and a combination of IBA and NAA, and the control treated with 50% aqueous solution of ethanol. Results obtained showed that rooting in cladodes is most effective when treated with 2500 mg L<sup>-1</sup> of IBA. A non-significant effect on rooting was observed when cladodes were treated with NAA alone or in combination with IBA. The development of *O. ficus-indica* in orchard and farmlands particularly in the dry Sudano-Sahelian zone of Nigeria would provide a valuable source of vegetarian food for humans and feed for livestock.

**Key words:** *Opuntia ficus-indica*, rooting, cladodes, Indole butyric acid (IBA) Naphthalene acetic acid (NAA).

## INTRODUCTION

Cactaceae is a family of perennials with high efficiency in converting water to dry matter due to its characteristic photosynthetic pathway of Crassulacean Acid Metabolism (CAM), surpassing C-4 plants such as corn by four to five-fold. Cactus pear (*Opuntia ficus-indica*) has become an important crop with great potentials of ensuring sustainable and valuable agricultural production in arid and semi-arid regions of the world. Its products are very popular among rural population in arid and semi-arid areas in its native region of Mexico and in the Mediterranean basin (Barbera, 1995). Cladodes and fruits are useful for a variety of purposes including food, fodder, therapeutics and industrial products (Nobel,

1994). In the last decades, an increase in the demand of tropical fruits has led to establishment of cactus orchards for fruit production in many countries (Synman, 2006). *Opuntia* was reported to be one of the most widespread cacti, primarily due to their edible fruit and vegetable mass used as feed (El-Finti et al., 2013).

Despite the large range of commodities it could provide in areas with scarce available resource, cactus has received little attention in Nigeria. However, recently the cultivated spineless cactus pear was introduced to Jigawa state (Anonymous, 2002). Although cactus pear can be propagated from seeds, cladodes and other asexual methods such as grafting and tissue

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culture (Estrada-Luria et al., 1994), based on whole cladodes are more commonly used in conventional propagation practices. Vegetative propagation relies on the plant's ability to produce new roots and shoots from an existing part of the plant such as a stem or leaf (Kelly, 2009). While the establishment of vast plantations of *Opuntia* requires large quantities of propagated materials, commercial scale propagation of Cactus using tissue culture techniques is posed with a serious problem due to unreliable public utilities in Jigawa State. This underscores the need for a simple technique that will ensure rapid on-farm propagation over a short period of time.

Root dynamics of *Opuntia* species have been shown to be related to their water potentials under water limiting conditions (Joseph et al., 1998; Synman, 2006). According to Synman (2006) placing the cladodes flat on the soil, more areoles came in contact with the soil and therefore more roots developed in both *O. ficus-indica* and *O. robusta* with an average of only 3.4% areole complexes not rooting. Side roots grew as much as 8 and 5 mm per day for *O. ficus-indica* and *O. robusta*, respectively. Rain roots, grew up to 7 and 5 mm within a day for *O. ficus-indica* and *O. robusta*, respectively. Plant growth regulators (PGRs) have also been shown to influence organ differentiation in *Opuntia* (Mauseth, 1976). Exogenous Gibberellin ( $GA_3$ ) inhibits the development of daughter cladodes as well as flower production in the plants (Nobel, 1996). On the other hand, Indole-3-butyric acid (IBA) and Naphthalene acetic acid (NAA) were shown to have a positive effect on root development and increase in cladode weight of *O. ficus-indica* (Mulas et al., 1992, Lazcano et al., 1999). Under *in vitro* condition, IBA was reported to produce more roots in *O. ficus-indica* and other species of *Opuntia* (Mohammed-Yasseem et al., 1995; Juárez and Passera, 2002). This work aims at enhancing root system development of *O. ficus-indica* cladodes as a means of facilitating its field establishment over a short propagation period.

## MATERIALS AND METHODS

The experiment was carried at Jigawa Research Institute, Kazaure (JRI), between the months of February to June 2013. JRI falls within the dry Sudano-Sahelian ecological zone of Nigeria. The cladodes used in this experiment had a mean length of 24.6 cm and a mean width of 14.1 cm and were obtained as mature secondary cladodes of the cultivated species of *O. ficus-indica*. Quick-dip solutions consisting of 2,500, 5,000, 7500 and 10,000  $mgL^{-1}$  of three auxin types; IBA, NAA and a 50:50 mixture of IBA and NAA were prepared with 50% aqueous solution of ethanol. Ten cladodes were used for each treatment including the control, which was treated with 50% aqueous solution of ethanol. Cladodes were treated by dipping 4cm of the basal part into the auxin solutions for four seconds. They were then planted in the field in a randomized complete block design with three replications. Water (equivalent to 300 mm annual rainfall) was applied to the experimental plots to maintain moisture. Rooting was observed after two weeks of establishment by carefully uprooting the cladodes without

damaging the roots. They were then washed with clean water and the number and lengths of roots were recorded. The root dry weight was obtained as the final dry weight after drying in oven at 75°C for 42 h. The data obtained was analyzed using SAS ANOVA procedure and means separation using Duncan Multiple Range Test (SAS Institute, 1988).

## RESULTS AND DISCUSSION

The soil of the study area is predominantly sandy with a pH of 8.5, low level of organic matter (1.04%) and high levels of Phosphorous (90.0 ppm) and Potassium (108 ppm). The prevailing climate at the time of the experiment is characterized by no rain, very low humidity and high day temperature of 35 to 39°C. Rooting was observed in the treatments, including the control and only 5% of the cladodes became putrid due to fungal infection. Analysis of variance in this experiment indicates that block effect was not a significant factor in rooting of cladodes. However, treatments with different levels of auxin had a significant effect in the rooting (Table 1). Root number, length and dry weight were significantly higher ( $p < 0.005$ ) in cladodes treated with IBA than in those treated with NAA or IBA+NAA. The result showed that greater root number, root length and root dry weight were obtained with 2500  $mg L^{-1}$  IBA. Treating cladodes with NAA or IBA+NAA did not significantly affect the root length and dry weight in cactus pear. There was some increase in the root number when cladodes were treated with NAA or IBA+NAA, with corresponding decrease in root length. The root length in control treatment was higher than root length in cladodes treated with NAA or IBA+NAA. Indole-3-butyric acid at 2500  $mg/L$  significantly increased root dry mass.

There were significant differences in rooting among IBA-treated and NAA-treated, IBA+NAA-treated and the untreated cladodes of *O. ficus-indica*. In the presence of IBA, cladodes rooted with high frequency, while the untreated cladodes rooted poorly and tended to produce fewer roots. The best result in terms of root number, root length and root dry weight was obtained with IBA at concentration of 2500  $mgL^{-1}$ . The findings of this experiment show that rooting response in cladodes of *O. ficus-indica* can be improved by applying exogenous IBA. Similar observation was reported by Lazcano et al., (1999) under field conditions. El-Finti et al., (2013) also reported that the rooting of *in vitro*-generated shoots was achieved most efficiently on half-strength MS basal medium supplemented with 0.5  $mgL^{-1}$  of indole-3-butyric acid (IBA) or IAA and with rooting frequencies were in the range from 95 to 100% and the highest mean number of root (19.1) was obtained with IBA. Currently IBA is the most widely used auxin to stimulate rooting in many plant species because of its high ability to promote root initiation (Nobel, 1996), mild toxicity alias great stability when compared with NAA and IAA. Although Auxins have been reported to stimulate enzymatic reactions and

**Table 1.** Effect of different concentrations of auxins on root system development in cladodes of Cactus.

Auxin Conc. (mgL <sup>-1</sup> )	Mean No. of Roots/cladode			Mean root length/cladode (cm)			Mean Root Dry Weight/Cladode (g)		
	IBA	NAA	IBA/NAA	IBA	NAA	IBA/NAA	IBA	NAA	IBA/NAA
*Control	3.3 <sup>d</sup>	3.6 <sup>d</sup>	3.5 <sup>d</sup>	3.3 <sup>bc</sup>	3.3 <sup>bc</sup>	3.2 <sup>bc</sup>	2.0 <sup>bc</sup>	2.0 <sup>bc</sup>	2.0 <sup>bc</sup>
2,500	26.7 <sup>a</sup>	8.3 <sup>bcd</sup>	8.9 <sup>bcd</sup>	6.3 <sup>a</sup>	2.5 <sup>c</sup>	2.6 <sup>c</sup>	5.1 <sup>a</sup>	2.5 <sup>bc</sup>	2.7 <sup>bc</sup>
5,000	19.1	8.0 <sup>bcd</sup>	8.0 <sup>bcd</sup>	4.8 <sup>ab</sup>	2.0 <sup>c</sup>	2.1 <sup>c</sup>	2.7 <sup>bc</sup>	2.6 <sup>bc</sup>	2.9 <sup>bc</sup>
7,500	14.7 <sup>b</sup>	7.5 <sup>cd</sup>	7.1 <sup>cd</sup>	4.5 <sup>b</sup>	2.0 <sup>c</sup>	2.1 <sup>c</sup>	2.7 <sup>bc</sup>	2.4 <sup>bc</sup>	2.5 <sup>bc</sup>
10,000	14.7 <sup>b</sup>	3.7 <sup>d</sup>	5.0 <sup>d</sup>	2.8 <sup>c</sup>	1.7 <sup>c</sup>	2.0 <sup>c</sup>	2.3 <sup>bc</sup>	1.9 <sup>bc</sup>	1.9 <sup>bc</sup>
SE (±)		0.96			0.62			0.63	

Means followed by same letter(s) within a parameter are NOT significantly different using Duncan. Multiple Range Test (p=0.05). \* = 50% aqueous solution of ethanol.

thus increase the rate and quality of root production in high concentration, they can have the opposite effect and retard or inhibit the formation of roots. The effectiveness of auxins can also vary among species of plant, thus the optimum concentrations and combinations of auxins may differ among species. Root dry weight decreased with increase in concentration of auxins from 5,000 mg/L to 10,000 mg/L. Although the number of roots in cladodes treated with NAA and IBA+NAA was not significantly (p<0.005) different from the control (Table 1), the rate at which the roots were produced as measured by their length was lower than control. NAA and IBA+NAA did not significantly increase root dry weight in cladodes of *O. ficus-indica*, and apparently a decrease in the root dry weight occurred along with increases in the concentration of auxins.

## Conclusion

IBA application at concentrations between 2500 to 10000 mgL<sup>-1</sup> has a positive effect on the root number of *O. ficus-indica*. The best rooting in terms of root number, root length and root dry weight was recorded with 2500 mgL<sup>-1</sup> of IBA. Treating cladodes with NAA and IBA+NAA at concentrations used in this experiment did not significantly increase root number, root length and root dry weight. Root length and dry weight decreased with increase in IBA concentration. Treatment with 2500 mg L<sup>-1</sup> IBA is an alternative technique for enhancing root system development that should be tested for the establishment of Cactus orchards and farms in semi-arid parts of Nigeria.

## Conflict of interests

The authors have not declared any conflict of interests.

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