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Comparative Response of Cabbage to Irrigation in Southern Malawi

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Abstract

An experiment was conducted at Kasinthula and Masenjere in Chikwawa district in Malawi in the dry seasons (May through August) of 2006 and 2007 to evaluate yield response of cabbage (Brassica oleraceae) to irrigation frequency. The study was laid out in a randomized complete block design (RCBD) where three irrigation frequencies served as treatments: F1-Irrigated twice a week, F2-Irrigated once a week and F3-Irrigated once a fortnight. At Kasinthula, weight of marketable heads and water-use efficiency (WUE) were significantly different ($P<0.05$) across the irrigation frequencies. At Kasinthula and Masenjere, F1 resulted in highest yield of 32.9 and 23.0 t ha^{-1} in 2006 and 2007 seasons. There was a 50% and 25% reduction in yield in 2007 at Kasinthula and Masenjere Research sites. WUE peaked in F1 to 83.6 and 57.5 kg ha^{-1} mm^{-1} in 2006 and 2007 while lowest values were noted using F3 resulting in WUE of 57.9 and 39.4 kg ha^{-1} mm^{-1}. Water productivity (WP) was significantly different across irrigation frequency ($P<0.05$). F3 resulted in the highest WP of 11.8 and 7.4 kg m^{-3} in 2006 and 2007, respectively. The lowest WP of 6.7 and 5.2 kg m^{-3} were observed in F1 in the two years. Comparing all the irrigation frequencies, F3 turns out to be the most effective water saving irrigation frequency suggesting that in the face of competing water needs and dwindling water resources, the longer duration F3 irrigation frequency is preferred to shorter duration ones. Where water is considered ample, F1 is recommended.

Keywords: irrigation frequency, furrow irrigation, water productivity, water-use efficiency

1. Introduction

Vegetables are an important source of vitamins and mineral which are vital for good health. Despite being widely grown in Malawi, adequate supplies of vegetables are mostly available in the rainy season (November through April) yearly. Consequently, supplies are not adequate throughout the year especially in semi-arid areas of the country. By increasing water productivity in vegetable production, irrigated agriculture may increase substantially (FAO, 2000; Rockstrom et al., 2002). Smith (2000) reported that genetic characteristics of a crop are the primary factors determining water use productivity because they determine assimilation characteristics, the respiration and physiological processes that convert the assimilates into biomass and harvest index which partitions biomass into the harvestable product and non-useful product. Doorenbos and Kassam (1979) categorized crops according to their yield response to water namely: crops sensitive to water stress and those tolerant to drought. They observed that the crops are self-exclusive, meaning that high producing crops which can obtain high water productivity are highly sensitive to water stress, while stress tolerant crops can still achieve yields under water stress but do not attain the high yield and water use under optimal water supply, ceteris paribus.

The amount of water required by plants and the timing of irrigation are governed by prevailing climatic conditions, crop and stage of growth, soil moisture holding capacity and the extent of root development as determined by crop type, stage of growth and soil. Thus, the amount of water required by plants varies from place to place. For practical purposes, it is important to determine the relationship between yield and water in a given locality in a simplified form because the relationship is intrinsically complex (Doorenbos & Kassam, 1979) as it is affected by factors other than water such as crop variety, salinity, pests and diseases, and agronomic practices. Better irrigation management after determination of yield response could help in maximizing WUE and avoiding long-term build-up of salinity and soil degradation (Hess & Molatakgosi, 2009). Also, improved water management would help in coping with increasing demands for water by industrial and urban users and the agricultural sector (de Fraiture & Wichelns, 2010) thereby making water available for environmental and other uses (de Fraiture et al., 2010). De Fraiture et al. (2010) advocate for a fresh look at approaches that combine different elements such as the importance of adapting irrigation to new needs, enhancing water productivity, and promoting the use of low-quality water in agriculture. Several researchers observe that there are sufficient land and water resources available to satisfy global food demands during the next 50 years, but only if water is managed more effectively in agriculture.

In Malawi, no study has been conducted recently on vegetables such as cabbage to ascertain the crop's response to various irrigation regimes. It is important that on-farm and/or on-station water management studies are conducted on vegetables under various irrigation schedules and frequencies to determine water use patterns and yields for efficient irrigation management. This explains the need for irrigated vegetable production to meet both subsistence needs of farmers and their commercial objectives. Also, through schedules developed in the studies, it will be possible to provide resource-poor farmers in semi-arid areas of Malawi with options for supplementary irrigation in summer (rainy season) and full irrigation in winter (dry season). Improved irrigation management appears to be the panacea to increased cabbage availability and production in Malawi.

The experiment was conducted to evaluate yield, water-use efficiency and productivity of cabbage under three irrigation frequencies. It was hypothesized that irrigation frequency does not have any significant impact on cabbage yield, water-use efficiency and water productivity.

2. Method

2.1 Study Sites

The experiment was conducted at Kasinthula and Masenjere in Chikwawa District in southern Malawi. Kasinthula lies at 16^{o}S 34^o 5′E at 60 m above sea level (a.s.l.) (Fandika et al., 2007) while Masenjere is located at 16°20'S, 35°6'E, approximately 93 a.s.l (Poynton, 1995). The soil at Kasinthula Research Station is Kapasule series, provisionally classified as aquic Hapslustalf (Barak, 1986) in the order Alfisols. The surface horizon is loamy sand while the subsurface horizon is sandy clay loam. It is moderately coarse to moderately fine textured developed in the brown sediments of the lower Shire terrace. The soil is moderately drained with a water table at 2.5 m from the ground surface. The available was estimated to be 100 mm m^{-1} (Chilimba, 1990). Soil physical and chemical characteristics for Kasinthula and Masenjere are presented in Table 1.

The average climatic conditions of Kasinthula Experiment Research Station monthly total rainfall (mm), average maximum and minimum temperature (^{o}C), relative humidity (%), wind speed (km hr^{-1}), sunshine hours (h d^{-1}), solar radiation (Langley), and monthly total evaporation (mm) are presented in Table 2 below. Masenjere research site receives average annual rainfall of 860 mm. The soil is a deep, colluvial sandy loam (Poynton, 1995).

Table 1. Average soil characteristics at Kasinthula and Masenjere

Soil depth (cm)	Sand (%)	Silt (%)	Clay (%)	Textural class	FC (%)	PWP (%)	Available water (%)	Bulk density (g/cm^3)	pH (water)	N (mg/kg)	P (mg/kg)
						Kasinthula					
0-30	75	7	18	LS	10.27	5.65	4.62	1.67	6.44	6.0	24
30-60	74	7	19	LS	12.34	5.98	6.4	1.71	6.66	6.0	25
60-90	74	6	20	SCL	13.75	6.69	7.06	1.69	6.57	5.0	25
						Masenjere					
0-30	70	6	24	SCL	ND	ND	ND	ND	6.98	1.0	10
30-60	76	10	16	SL	ND	ND	ND	ND	7.03	1.0	13
60-90	80	8	12	SL	ND	ND	ND	ND	7.02	3.0	16

Notes: FC-Field capacity moisture content, PWP-Permanent wilting point, ND-Not determined.

Table 2. Meteorological data during the dry seasons at Kasinthula in 2006 and 2007

Weather variable	2006					
	May	June	July	August	September	October
Average maximum temperature (°C)	33.4	29.2	28.9	29.7	33.6	35.3
Average minimum temperature (°C)	18.3	15.0	12.7	17.5	21.5	22.0
Average relative humidity (%)	64.0	68.2	71.3	64.0	57.4	78.4
Average wind speed (km h^{-1})	3.7	3.4	2.2	2.9	5.9	4.6
Average sunshine hours d^{-1}	7.8	7.7	7.7	9.7	9.0	9.1
Total rainfall, mm	0.2	0.3	0.0	0.0	0.0	0.0
Average solar radiation (Langley)	476.0	453.0	474.0	512.0	537.0	573.0
Average pan evaporation (mm d^{-1})	4.6	4.3	4.7	6.4	7.9	9.7
	2007					
Average maximum temperature (°C)	30	26.5	22	31.9	34.7	35.6
Average minimum temperature (°C)	17	14.1	13	13.3	15.6	19.8
Average relative humidity (%)	81	91	71	89.6	92.2	72
Average wind speed (km h^{-1})	7	8.9	11	11.9	16.1	19.2
Average sunshine hours d^{-1}	8	8.5	8	8.9	9.7	9.9
Total rainfall, mm	4.9	5.1	45.2	6.4	0	14
Average solar radiation (Langley)	-	-	-	-	-	-
Average pan evaporation (mm d^{-1})	4.9	5	3.8	5.4	7.2	8.9

2.2 Experimental Design and Treatments

Irrigation frequencies constituted the treatments in the study namely: F1-Irrigated twice a week, F2-Irrigated once a week, and F3-Irrigated once bi-weekly. All treatments were replicated four times, in plots 5 m by 5 m, where 1 m was left on each side as a buffer for gravimetric soil moisture determination and routine measurements. Plants, cabbage variety Giant drumhead, were planted at 0.6 m in between and within rows.

2.3 Fertilizer and Water Application

Calcium Ammonium Nitrate (CAN), an inorganic fertilizer was applied at the rate of 100 kg N ha^{-1} by banding. Irrigation water was applied in 5-m long furrows using a gated 10-cm diameter PVC pipe. Each gate was set at a flow rate of 30 litres per minute. The flow rate out of each gate was determined using a calibrated bucket. The PVC pipe was laid at the beginning of the furrows, and connected to a concrete lined canal in which water level was maintained at a constant head above the center of the PVC pipe inlet.

2.4 Irrigation Scheduling

The experimental plots were irrigated to field capacity at planting and irrigation schedules were imposed four weeks after planting. The soil moisture storage was estimated from available water holding capacity of 100 mm m^{-1} and the crop root zone. The crop consumptive use was computed from climatic data using the evaporation pan method for estimating reference evapotranspiration (ETo). The ETo was calculated daily and then multiplied by the cabbage crop coefficient (Kc) (Table 3) at a particular growth stage to determine the consumptive water use based on well established procedures (Doorenbos & Pruitt, 1977; Doorenbos & Kassam, 1979; 1986; Allen et al., 1998), according to the following equation:

$$ETC = Kc * ETo \tag{1}$$

Where ETc is crop consumptive water use (mm).

The equation for estimating crop WUE (kg ha^{-1} mm^{-1}) according to Kirda (2002) and Lovelli et al. (2007) is:

$$WUE = \frac{Y}{ETa} \tag{3}$$

Where Y is crop yield (kg ha^{-1}) and ETa is actual evapotranspiration (mm) which was regarded as crop evapotranspiration (mm), in this study.

Water productivity (WP in kg m^{-3}) according to Smith (2000) is given by the following expression:

$$WP = \frac{Y}{TWA} \quad (3)$$

Where TWA is total water applied (mm).

Table 3. Crop coefficients (Kc) for cabbage at various growth stages

Growth stage	Crop coefficient, Kc	Days after planting (DAP)
Initial stage	0.45	1
Crop development stage	0.75	22
Mid-season stage	1.05	48
Late season stage	0.9	118

2.5 Statistics

All the data were analyzed in GENSTAT 6.0 and means were separated using the Fisher's Least Significant difference (LSD) method.

3. Results and Discussion

3.1 Cabbage Yield at Kasinthula and Masenjere

Irrigation frequency significantly influenced weight of marketable heads at both sites ($P<0.05$). At Kasinthula (KAS) and Masenjere (MAS), F1 resulted in highest yield of 32.9 and 23.0 t ha^{-1} in 2006 and 2007 seasons (Figure 1). There was a 50% and 25% reduction in yield in 2007 at Kasinthula and Masenjere research sites. In both years, yields were four to five times higher at Masenjere than Kasinthula. Also, number of marketable heads at Masenjere was two to three times higher compared with that of Kasinthula. The results show that the Giant Drumhead variety of cabbage favours Masenjere and may not be suitable for Kasinthula.

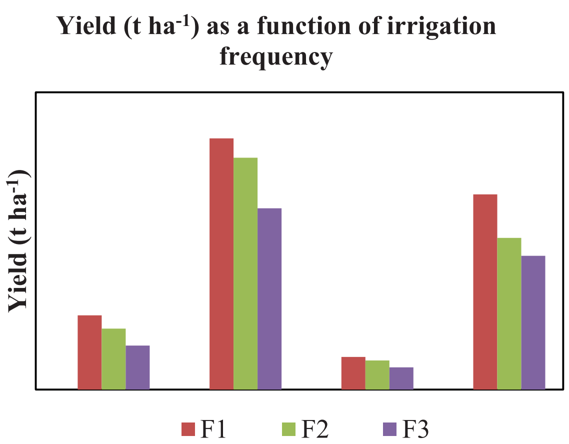

Figure 1. Yield (t ha^{-1}) as a function of irrigation frequency

However, there were no significant differences in yields and number of marketable heads at Masenjere in both years between the three irrigation frequencies (Figure 2). This suggests that farmers faced with dwindling water resources farmers could opt for irrigation frequency F3 where 202 mm of water was applied to cabbage. Approximately 508 and 306 mm of water were applied in irrigation frequencies F1 and F2. F3 offers the growers a rational basis realizing reasonable yield while saving water. Where a farmer has ample water resources for the entire season, the F3 would still be employed but with adjustments on the amounts of water to be applied per season. F3 present an opportunity for introducing deficit irrigation in cabbage at Kasinthula and Masenjere. It was noted that average cabbage evapotranspiration for Kasinthula and Masenjere was 393 mm and was exceeded by F1 and F2. F3 did not meet the cabbage water requirement.

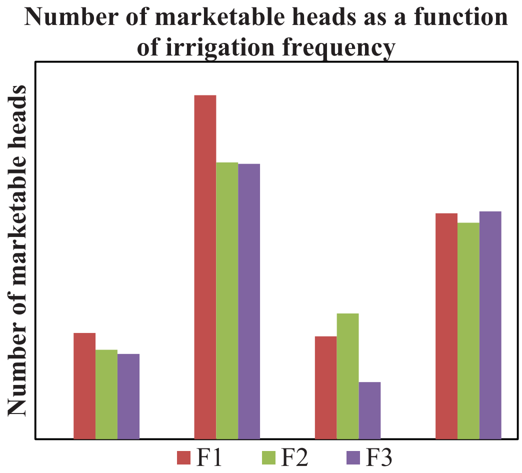

Figure 2. Number of marketable heads as function of irrigation frequency

The results agree with the findings of several researchers (Kleinhenz & Radovich, 2003; Radovich, 2004; Radovich et al., 2004) who documented the effect of irrigation timing on head development and weights on cabbage. They concluded that head size and weight were greatest in cabbage receiving irrigation during head development. In their studies, the independent and interactive effects of year and irrigation treatment were largely explained by the proportion of crop evapotranspiration replaced during head development. The yields obtained in Masenjere are similar to those reported by Imtiyaz et al. (2000) in field studies in Botswana where cabbage yielded as high as 71.65 t ha^{-1}. The cabbage yields at Kasinthula, despite receiving similar irrigation management treatments, suggest that the variety Giant drumhead is not suitable for the area.

3.2 Water Use Efficiency (WUE) and Productivity (WP) of Cabbage

WUE was significantly different across irrigation frequencies ($P<0.05$) at Kasinthula only in both years (Table 3). WUE peaked in F1 to 83.6 and 57.5 kg ha^{-1} mm^{-1} in 2006 and 2007 while lowest values were noted using F3 resulting in WUE of 57.9 and 39.4 kg ha^{-1} mm^{-1}. WUEs at Masenjere were four to five times those obtained at Kasinthula research site probably due to high evaporative demand at Kasinthula compared with Masenjere site (Barak, 1986).

Table 3. Effects of irrigation frequency on water-use efficiency (kg ha^{-1} mm^{-1})

¶Irrigation frequency	2006		2007	
	Kasinthula	Masenjere	Kasinthula	Masenjere
F1	38.1a	129.1a	16.4	98.6
F2	31.3b	119.0a	14.7	76.6
F3	22.7c	93.1b	11.2	67.5
‡$LSD_{0.05}$	4.7	15.0	4.6	13.9
†CV (%)	17.3	14.8	15	12.8
§Significance	*	NS	*	NS

¶F1-Irrigated twice a week, F2—Irrigated once a week and F3-Irrigated once a fortnight.

‡Least significant difference at α=0.05.

†Coefficient of variation.

§NS, * *mean not significant and significant at* α=0.05.

Water productivity was significantly different across irrigation frequency ($P<0.05$). F_3 resulted in the highest WP of 11.8 and 7.4 kg m^{-3} in 2006 and 2007, respectively. The lowest WP of 6.7 and 5.2 kg m^{-3} were observed in F_1 in the two years. The results on WP show when faced with competing water needs in irrigated crop production then longer irrigation schedules are preferable. Our results on WP in vegetables agree with those obtained by Pachpute (2010) who reported water productivity of 12.1 kg m^{-3} in cucumber using pitcher irrigation and water conserving technologies such as manure application and mulching in neighboring Tanzania. The WP values also fall within the ranges reported by Molden et al. (2010) for vegetable crops. Comparing all the irrigation frequencies, F_3 turns out to be the most effective water saving irrigation frequency suggesting that in the face of competing water needs and dwindling water resources, the longer duration F_3 irrigation frequency is preferred to shorter duration ones.

Table 4. Effects of irrigation frequency on water productivity (kg m^{-3})

¶Irrigation frequency	2006		2007	
	Kasinthula	Masenjere	Kasinthula	Masenjere
F1	3.4b	10.0a	3.2	7.2
F2	3.7ab	13.8b	5.2	8.9
F3	4.4a	18.1c	2.9	11.8
‡$LSD_{0.05}$	1	3	2.5	4.3
†CV (%)	30.2	21	13.3	21.1
§Significance	NS	*	NS	*

¶F1-Irrigated twice a week, F2—Irrigated once a week and F3-Irrigated once a fortnight.

‡Least significant difference at α=0.05.

†Coefficient of variation.

§NS, * *mean not significant and significant at* α=0.05.

4. Conclusion

The study sought to determine the effect of irrigation frequency on WUE, WP and yield of cabbage. As confirmed in the results, we conclude that irrigation frequency has a significant bearing on yield, WUE and WP of cabbage. We also note that research site resulted in remarkable differences in the crop response functions under study. Thus, we suggest further investigations using several cabbage varieties at both Kasinthula and Masenjere research sites before passing on the recommendations to farmers in order to obtain more consistent results. However, some clear trends were observed. First, F1 resulted in highest yield and WUE in both seasons while F3 yielded lowest. The reverse was true for WP. Comparing all the irrigation frequencies, F3 turns out to be the most effective water saving irrigation frequency suggesting that in the face of competing water needs and dwindling water resources, the longer duration F3 irrigation frequency is preferred to the shorter duration ones. Where water is considered ample, F1 is recommended. Resources permitting, a research attempt on raising cabbage under both controlled greenhouse and field conditions should be made because field studies alone consistently showed low cabbage yields at Kasinthula despite good irrigation management.

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Chlorophyll Fluorescence Transient Analysis in *Alternanthera tenella* Colla Plants Grown in Nutrient Solution with Different Concentrations of Copper

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Abstract

This study evaluates the chlorophyll index and fluorescence parameters of chlorophyll *a* in *Alternanthera tenella* grown in different concentrations of copper (Cu). The *A. tenella* plants were hydroponically grown in a complete nutrient solution for 3 days and were then transferred to the same solution containing different copper concentrations [0.041, 0.082 (control), 0.164, 0.246, and 0.328 mM]. The plants were maintained in these solutions for 13 days. The nutrient solutions were replaced every 3 days, and the plants were evaluated at 6 days and 13 days after the start of the treatment. An increase in the chlorophyll index was observed at copper concentrations of 0.164, 0.246, and 0.328 mM on days 6 and 13. These concentrations of copper also affected the fluorescence parameters and caused a reduction in the pool of the final photosystem I (PSI) electron acceptors, a decrease in the total number of electron carriers per reaction centre, a decrease in the parameters related to the flow, yield, and efficiency for the reduction of the final PSI acceptor, and a decline of the total photosynthetic performance index. Therefore, high doses of copper affect the functionality of the PSI, decreasing the reduction flow of the final PSI electron acceptors.

Keywords: plant nutrition, photosynthesis, micronutrient, copper sulphate, JIP-Test

1. Introduction

The recent increase in agricultural and industrial activities has contributed to the increased occurrence of heavy metals in the ecosystem. However, plants need minerals, which are mainly acquired from the soil, to maintain normal growth and development and to ensure the completion of their life cycles (Yruela, 2009). Copper (Cu), a transition metal, is an essential micronutrient for plants and exists in two oxidation states in the cellular environment, Cu^{2+} and Cu^{+} (Yruela, 2005). Copper can be absorbed by the roots of the plant in the form of chelated copper complexes (citric acid, tartaric acid, malic acid, oxalic acid, metallophores, phenols, etc.) and as Cu^{2+}. The translocation of copper to the shoot occurs via the xylem, through the transpiration chain, and for the most part, the copper is complexed with amino acids (Prado, 2008). Inside the cell, copper is required at several locations, such as the cytosol, endoplasmic reticulum, mitochondria, chloroplasts, and apoplasts (Yruela, 2009). Copper also plays a variety of roles in plant metabolic processes including photosynthesis, respiration, antioxidant activity, the biosynthesis of cell wall components, lignification, ethylene detection, and the production of secondary metabolites (Pilon et al., 2006; Burkhead et al., 2009; Lequeux et al., 2010; Perotti et al., 2010).

The majority of the copper in the plant is located in the chloroplasts of the leaves, and more than half this ion is bound to plastocyanin, which is a Cu-containing protein that participates in the transport of electrons between photosystems II and I (PSII and PSI). This electron transport mechanism is affected in plants deficient on the copper (Prado, 2008). The photosynthetic apparatus is sensitive to this cation, and the in the low or high level leads to changes in the function of PSII, the rate of electron transfer, and the formation of plastocyanin (Yruela, 2009; Cambrollé et al., 2011).

Considering the importance of photosynthesis for the growth and development of plants, and the sensitivity of the photosynthetic apparatus to environmental stress, the measurement of chlorophyll *a* fluorescence is an important technique for assessing photosynthetic efficiency, specifically for the behaviour of PSII (Mehta et al., 2010; Buonasera et al., 2011; Yang et al., 2012). The use of this direct, non-destructive, highly sensitive, and reliable technique has provided a greater understanding photosynthetic process (Rohácek, 2002). This technique allows the study of characteristics related to the absorption and transfer of light energy in the electron transport chain in chloroplast (Krause & Weis, 1991).

Evaluating the chlorophyll *a* fluorescence allows the calculation of the parameters that estimate the energy absorption by the pigments of the antenna system, the capture of an exciton by the reaction centre, and the subsequent electron transport to the final electron acceptor (Yusuf et al., 2010). From the measurement of the fluorescence transient (OJIP) using the JIP-Test (Strasser & Strasser, 1995), it is possible to quantify the flux of energy passing through the photosystems, to evaluate the photosynthetic performance of plants, and to analyse the PSII operation (Strasser et al., 2004; Tsimilli-Michael & Strasser, 2008).

Small concentration and excess of the copper in plant can cause nutritional problems that adversely affect physiological processes, numerous studies have investigated the effect of different concentrations of this element, with particular emphasis on the energy flux and the electron transport chain. This study measured the chlorophyll index and the parameters of the chlorophyll *a* fluorescence transient in *Alternanthera tenella* plants grown in nutrient solutions containing different copper concentrations.

2. Material and Methods

2.1 Plant Material and Experimental Conditions

Alternanthera tenella Colla plants were established and multiplied *in vitro* and were acclimatised for 2 weeks in a growth chamber under a photosynthetically active photon flux density of 120 μmol m^{-2} s^{-1} and a 16-hour photoperiod. Subsequently, the plants were transferred to a continuous-flow root floating hydroponic system and were cultured using Hoagland and Arnon (1938) complete nutrient solution for 3 days. On the fourth day, the plants were transferred to the same nutrient solution containing the copper concentrations 0.041, 0.082, 0.164, 0.246, and 0.328 mM, provided in the form of copper sulphate ($CuSO_4 \cdot 5H_2O$). These concentrations were chosen according to preliminary tests with other plant species, which showed that the cultivation until the copper concentration of 0.082 mM did not show physiological changes (data not shown). Then, the concentration of 0.082 mM copper was employed as control. The plants were maintained under the same conditions of photon flux density and photoperiod for further 13 days. The solutions were changed every 3 days, and the chlorophyll *a* fluorescence transient and chlorophyll content were measured on days 6 and 13 after the start of the treatment (after transfer to solution with different copper concentration).

2.2 Chlorophyll Index and Chlrophyll a Fluorescence Transient

The chlorophyll content was estimated using a CL-01 portable chlorophyll meter (Hansatech Instruments Ltd., King's Lynn, Northfolk, UK), and the results are expressed as the "chlorophyll index" (Cassol et al., 2008).

The chlorophyll *a* fluorescence transient was measured in fully expanded leaves from 16 plants grown at each copper concentration, using a Handy-Pea portable fluorometer (Hansatech Instruments Ltd., King's Lynn, Norkfolk, UK). The measurements were performed on non-detached leaves that were previously adapted to the dark for 30 minutes for the complete oxidation of the photosynthetic electron transport system, and the fluorescence intensity was measured for 1 s after the application of a saturating light pulse of 3,000 μmol m^{-2} s^{-1}. The JIP-Test parameters were calculated based on the fluorescence intensities at 50 μs (minimum fluorescence), 100 μs, 300 μs, 2 ms (F_J), 30 ms (F_I), and F_M (maximum fluorescence) (Strasser & Strasser, 1995). The kinetic analysis of the chlorophyll *a* transient was evaluate by variable fluorescence between the steps OK, OJ and OI, by normalisation as follows: $W_{OK} = [(F_t - F_0)/(F_K - F_0)]$, $W_{OJ} = [(F_t - F_0)/(F_J - F_0)]$, and $W_{OI} = [(F_t - F_0)/(F_I - F_0)]$, respectively. Additionally, the differences between the ΔW_{OK} and ΔW_{OJ} transients relative to the control treatment (0.082 mM) were assessed as $\Delta W_{OK} = [W_{OK(stress)} - W_{OK(control)}]$ and $\Delta W_{OJ} = [W_{OJ(stress)} - W_{OJ(control)}]$ (Yusuf et al., 2010).

2.3 Statistical Analysis

The experiment was performed using a completely randomised design consisting of five doses of copper (0.041, 0.082, 0.164, 0.246, and 0.328 mM) and four replicates (four plants per replicate). The response variables were evaluated at twice (on days 6 and 13) during exposure to the copper stress. The experiment was repeated twice.

The chlorophyll index was subjected to analysis of variance (ANOVA), and the means were compared using Tukey's test at 5% significance.

3. Results

3.1 Chlorophyll Index

At 6 and 13 days of the stress treatment we observed that the chlorophyll index were more high in plant treated with higher doses of copper (Figure 1), and when we reduced the copper concentration for 0.041 mM Cu, the chlorophyll index not differed of the concentration control (0.082 mM).

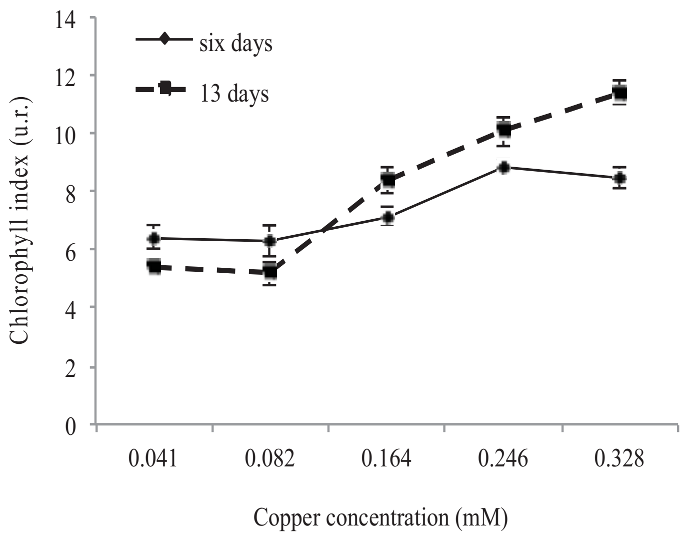

Figure 1. Chlorophyll index (relative unit) of *Alternanthera tenella* Colla plants subjected to different concentrations of Cu on days 6 (--♦--) and 13 (--■--) of the stress treatment. *Bar* indicates the standard error of the mean (n = 16)

3.2 Chlorophyll a Fluorescence Transient: Normalisation and Subtraction of Transients (OJIP)

The different copper doses had no marked effects on the chlorophyll *a* fluorescence *transient* (Figure 2A-2B) or on the relative variable fluorescence between steps O and P [($V_{OP} = (F_t - F_0)/(F_M - F_0)$] (Figure 2C-2D) obtained on days 6 and 13 after the start of the experiment. The normalisation and subtraction of transients between steps OK and OJ allows the verification of the appearance of the K-band (approximately 300 µs) and L-bands (approximately 150 µs). In present study the copper had no effect on the relative variable fluorescence between steps O (50 µs) and K (300 µs) or steps O (50 µs) and J (2 µs); thus, the K- and L- bands were not visualised (data not shown).

To investigate the effect of the different copper doses on the fluorescence transient IP phase, the following procedures, proposed by Yusuf et al. (2010), were followed: a) the interpretation of the relative variable fluorescence curve between steps O and I with values greater than or equal to 1 ($W_{OI} \geq 1$) (Figure 3A-3B), and b) the analysis of the normalised transients between steps I and P as relative variable fluorescence [$W_{IP} = (F_t - F_I)/(F_M - F_I)$] represented on a linear scale between 30 and 330 ms (Figure 3C-3D). On days 6 and 13, the size of the final PSI electron acceptor pool decreased at the higher copper doses (Figure 3A-3B). On day 6, the results from the lowest treatment dose were similar to the control dose, whereas on day 13, the size of the final PSI electron acceptor pool was inversely proportional to the dose, i.e., lower doses produced a larger pool (Figure 3B). Yusuf et al. (2010) proposed that for each curve, the maximal amplitude of the fluorescence rise reflects the size of the pool of the end electron acceptors at the PS I acceptor side. In other hand, the estimated the overall rate constant (inverse of the half-time - i.e., the inverse of the time to reach a $W_{IP} = 0.5$) of the PSI electron acceptors, did not show differences among the copper doses at the two periods analysed (Figure 3C-3D).

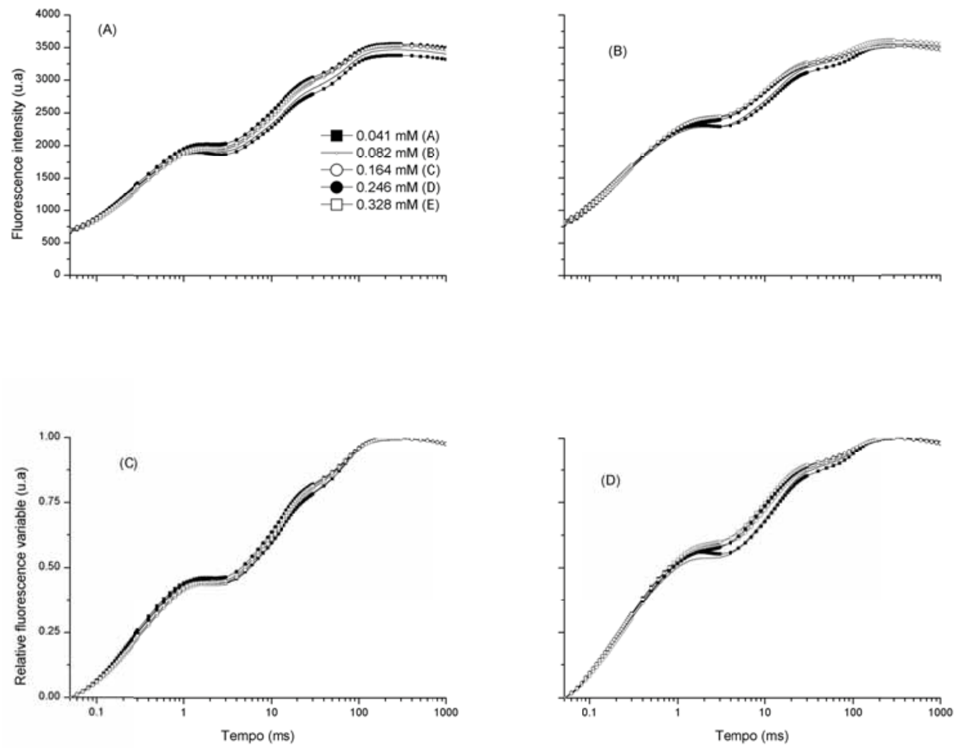

Figure 2. Chlorophyll *a* fluorescence transient of dark-adapted leaves of *Alternanthera tenella* Colla plants subjected to different Cu concentrations. A, B - Fluorescence intensity (F_t); C, D - Relative variable fluorescence [$V_{OP} = (F_t - F_0)/(F_M - F_0)$]. Plants were subjected to stress for 6 (A, C) and 13 (B, D) days

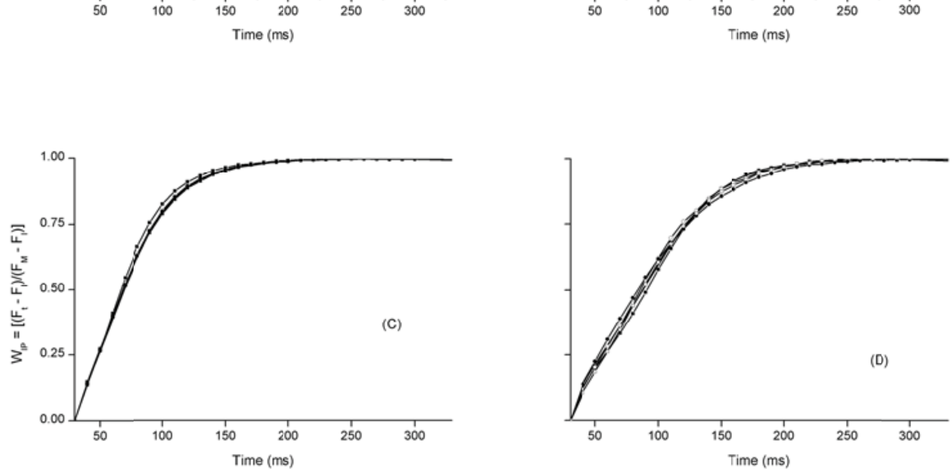

Figure 3. Chlorophyll *a* fluorescence transient of dark-adapted *Alternanthera tenella* Colla subjected to different Cu concentrations. A, B - Variable fluorescence between steps O and I [$W_{OI} = (F_t - F_0)/(F_I - F_0)$] in the 30 to 330 ms time interval; C, D - Variable fluorescence between steps I and P [$W_{IP} = (F_t - F_I)/(F_P - F_I)$]. Plants were subjected to stress for 6 (A, C) and 13 (B, D) days

3.3 Chlorophyll a Fluorescence Transient: PSII Biophysical Parameters Derived from the JIP-test Equations

The JIP-Test parameters are shown in the form of a radar chart, and all of the fluorescence parameter values were normalised to the values determined by the values for plants grown under the control treatment (0.082 mM Cu) on days 6 and 13 of the stress treatment (Figure 4A-4B).

The stress did not affect the normalised total complementary area above the transient curve only for the OJ phase (S_s), which reflects single-turnover Q_A reduction events (Chen et al., 2011). In contrast, lower value in the normalised total complementary area above the transient curve ($S_m = EC_0/RC$) was observed for the treatments with copper concentrations above 0.164 mM on day 6 and 13 of the stress, but the reduction was most obvious on 7 days. A similar effect was observed for the number of reduction turnovers, oxidation and re-reduction of Q_A in the time between turning on the light and reaching the F_M (N).

The specific flux (flux per active PSII reaction centre) of absorption (ABS/RC), trapping (TR_0/RC) and electron transport (ET_0/RC) showed little reduction compared to the control on days 6 and 13 of the stress treatment (Figure 4A-4B). However, the reduction flux of the final PSI electron acceptors (RE_0/RC) was the parameter that exhibited the greatest reduction above the control (0.082 mM) both on day 6 and 13 of the stress. At 13 days we observed that the plants exposed to 0.041 mM Cu exhibited an RE_0/RC that was greater than the control (Figure 4B).

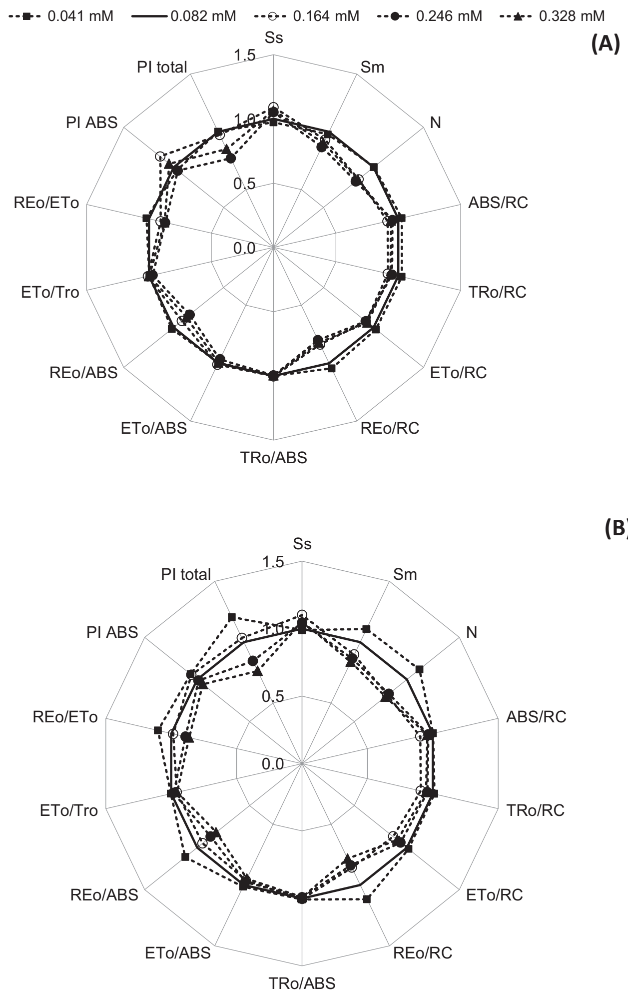

Figure 4. Chlorophyll *a* fluorescence parameters deduced from the JIP-Test analysis of fluorescence transient of *Alternanthera tenella* Colla plants subjected to different Cu concentrations (0.041, 0.082, 0.164, 0.246, and 0.328 mM). Plants were subjected to stress for 6 (A) and 13 (B) days

The quantum yields data demonstrated that a) the maximum photochemical quantum yield (φ_{Po}= TR_0/ABS) and the quantum yield of the electron transport from Q_A^- to the intersystem of electron acceptors (φ_{Eo} = ET_0/ABS) were not affected by the copper doses or by the period of stress, and b) the quantum yield for the reduction of the final PSI electron acceptor per photon absorbed (φ_{Ro} = RE_0/ABS) followed that observed for the RE_0/RC. The efficiency parameters did not demonstrate differences in the ψ_{Eo} = ET_0/TR_0 (probability/efficiency in which an exciton captured in the reaction centre can move an electron from the Q_A^- to the intersystem of the electron acceptors); however, the δ_{Ro} = RE_0/ET_0 (efficiency/probability that an electron of the intersystem electron carriers moves to reduce the final PSI electron acceptors or the likelihood of the reduction of a final PSI acceptor) decreased in the plants subjected to copper concentrations higher than the control on day 6 of the stress (Figure 4A) and in plants subjected to 0.246 and 0.328 mM Cu on day 13 of the stress (Figure 4B). However, the plants treated with 0.041 mM Cu exhibited a higher RE_0/ET_0 compared to the control on day 13 of the stress.

On day 6 of the stress treatment, the performance index based on the absorption (PI_{ABS}) (Strasser et al., 2004) showed a slightly higher value in the plants exposed to 0.164 mM Cu, whereas the other doses of copper did not induce changes in this parameter at this time point (Figure 4A). No differences in the PI_{ABS} were observed on day 13 of the stress treatment (Figure 4B). However, the analysis of the total photosynthetic performance index (PI_{total}), which measures the performance of electron flux to the final PSI electron acceptors (proposed by Tsimilli-Michael & Strasser (2008)), demonstrated that i) a reduction of the PI_{total} was observed on day 6 of the stress treatment at doses above the control and ii) on day 13 of the stress treatment, the plants exposed to 0.041 mM Cu exhibited an increase in the PI_{total}, whereas the plants grown at higher doses of Cu (0.246 and 0.328 mM) exhibited a reduction in the PI_{total}.

4. Discussion

The copper is an essential micronutrient for plant growth and is a component of many proteins, especially those involved in the photosynthetic (plastocyanin) and respiratory (cytochrome oxidase) electron transport chains (Pilon et al., 2006). However, high concentrations of copper are potentially toxic to most plants and the effects of this element on biochemical and physiological functions have been studied extensively. It has been recognised that copper toxicity induces the inhibition of the photosynthetic processes (Cambrollé et al., 2011).

The effect of metal ions on the photosynthetic pigments in algae and higher plants has been addressed in several studies; however, it has often proven difficult to distinguish the indirect effects of the elements on the plant metabolism. A reduction in the chlorophyll content with higher concentrations of copper and iron has been reported by Mourato et al. (2009) in yellow lupine (*Lupinus luteus* L.) and by Adamski et al. (2011) in sweet potato (*Ipomoea batatas* L.), respectively. This effect on the chlorophyll content is common for metal ion toxicity in plants. In this study, the copper had an inverse effect on the chlorophyll index, which is a widely used method for estimating the photosynthetic potential of plants because of the direct connection between the absorption and transfer of light energy, growth and adaptation to different environments (Almeida et al., 2004). Based on the association of the chlorophyll index values with the growth of plants in the presence of high doses of copper, it is possible that the increased concentration of this micronutrient enhanced the chlorophyll index values by decreasing the leaf area and the fresh and/or dried leaf tissue (data not shown) at the two evaluated time points, as shown by McMahon and Kelly (1990).

The use of chlorophyll *a* fluorescence in dark-adapted leaves has provided a greater understanding of the photosynthetic apparatus and its efficiency (Strasser et al., 2000). The chlorophyll *a* fluorescence transient curves in the plants exposed to different concentrations of copper (Figure 2A-2B) demonstrated a typical OJIP curves. The analysis of the transient kinetic differences between steps O and K (ΔW_{OK}) and steps O and J (ΔW_{OJ}) did not result in the appearance of the L- and K- bands, respectively. The failure to identify the L band suggests the maintenance of the energetic connectivity (grouping) of the PSII units, as demonstrated by Strasser and Stirbet (1998), and taking into account that the K- band was not identified, it can be inferred that the oxygen-evolution complex was not inactivated, as demonstrated by Yusuf et al. (2010).

The analysis of the IP phase transient (Figure 3A-3B) demonstrated that the effect of stress (high doses of copper) was reflected by the decline in the size of the electron acceptor pool of the PSI acceptor site (Redillas et al., 2011a). However, when comparing the transient analyses W_{OI} (Figure 3A-3B) and W_{IP} (Figure 3C-3D), it was observed that the decrease in the pool size was not associated with the regulation of the overall reduction rate of the PSI electron acceptors, suggesting that the regulation of these two parameters is independent. A similar response was observed in *Brassica juncea* plants overexpressing the α-tocopherol methyltransferase gene and treated with NaCl, $CdCl_2$, and mannitol (Yusuf et al., 2010) and in rice plants (*Oryza sativa* cv. Nipponbare) grown under nitrogen-limited conditions (Redillas et al., 2011b).

The use of the JIP-Test permits the understanding of processes related to energy flux in the electron transport chain, and this test is commonly used to discriminate plants grown under normal conditions from those grown under stress conditions (Redillas et al., 2011b). The flux starts with the absorption of light by the pigments of the PSII antenna and ends with the reduction of the final PSI electron acceptors (Yusuf et al., 2010). In this study, the interpretation of the JIP-Test parameters associated with the fluorescence emission kinetic analysis, especially in the IP-phase, demonstrated that different copper concentrations induced variations in the phase of the electron transport chain related to the reduction of the final PSI electron acceptors.

The analysis of the JIP-Test parameters demonstrated that compared to the control, the plants grown with high doses of copper demonstrated a smaller total complementary area above the OJIP or a reduced ability to transport electrons per reaction centre ($S_m = EC_0/RC$), indicating that less energy is needed to close all of the reaction centres (Strasser et al., 2000). According to Stirbet and Govindjee (2011), the S_m is proportional to the number of electrons passing through the electron transport chain (i.e., multiple-turnover Q_A reduction events), with $N = S_m/S_s$ (where S_s only represents the complementary normalised area between the OJ phase, reflecting single-turnover Q_A reduction events), i.e., N is the number of times that Q_A becomes reduced and re-oxidised, until the maximum fluorescence F_M (i.e., the number of turns) is reached. Based on this equation, it was possible to show that the dose-dependent changes initiated in the PSII, i.e., for the evaluation of parameters describing the oxidation and reduction of the PSII electron acceptors (S_m and N).

Thus, the results show that the flux (RE_0/RC), the yield ($\varphi_{R0} = RE/ABS$), and the efficiency ($\delta_0 = RE_0/ET_0$) may have been the limiting factors in the flux of electrons that permit the reduction of plastoquinone in the PSII. It can be inferred that high doses of copper are toxic in plants, stimulating metal detoxification mechanisms, such as complexation (Hänsch & Mendel, 2009), thus limiting the availability of this element for the plastocyanin molecule, which is responsible for maintaining intersystem electron transport to the PSI. Together, the parameters of the chlorophyll fluorescence transient indicates that increasing the dose of copper reduced the photochemical and non-photochemical redox reactions in the plants subjected to copper stress for 6 and 13 days, which is also reflected by the reduction of the PI_{total}.

The performance indices (PI_{ABS} and PI_{total}) are reflect the conservation of energy from photons absorbed by the PSII antenna system; changes in these indices are considered good indicators of photosynthetic activity or even stress that directly or indirectly damages the photosynthetic apparatus in plants (Stirbet & Govindjee, 2011). The PI_{ABS} index provides complete and quantitative information regarding the vitality of plants (Strasser et al., 2004). A slight increase in the PI_{ABS} after 6 days of exposure to 0.164 mM Cu can be interpreted as an adaptation to the initial stress, i.e., a compensation for its low photosynthetic capacity (Oukarroum et al., 2009) and/or this can be attributed to the higher RC/ABS component values observed for these plants. The PI_{total} index has been described as the most sensitive parameter of the JIP-Test, measuring the partial energy conservation potential to the final PSI electron acceptors (Tsimilli-Michael & Strasser, 2008; Yusuf et al., 2010). According to Yusuf et al. (2010), a negative or positive PI_{total} expresses a “loss” or “gain”, respectively, of the energy conservation capacity, and this value is considered an efficient tool to quantify stress in plants. A decrease in the PI_{total} in plants subjected to high doses of copper not only resulted in the loss of PSII activity but also caused structural and/or functional damage to the PSI.

This study showed that *A. tenella* plants grown in high concentrations of copper did not exhibit differences in the capture and absorption of energy by the PSII; however, an impairment of the PSI related parameters was observed. The excess copper caused the following effects: a reduction in the size of the final PSI electron acceptor pool, a reduction in the total number of electron carriers per reaction centre, and a decrease in the parameters related to the flow, yield, and efficiency of reduction of the final PSI acceptors, as evidenced by the reduction of the PI_{total}. Therefore, the high doses of copper predominantly affect the structure and functionality of the PSI.

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The Effect of Ethrel Application on Length of the Juvenile Phase of Apple Seedlings

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Abstract

A 10 year study (1998-2007) was conducted to work out a method that would allow shortening of the juvenile phase of apple seedlings in conventional apple breeding programs. The investigation was based on two separate experiments, each conducted first in high plastic tunnel and then in the field. In both experiments in the first two years, the seedlings were grown on their own roots in plastic containers (cylinders) placed in the high plastic tunnels. At the end of the second year (December), shoot tips from the seedlings were collected and grafted onto dwarfing rootstock M.9 (winter grafting). By the end of March, the grafts were planted in plastic containers and placed in the same high plastic tunnel. They were cultivated there until mid - August and then planted out to the selection field. In the first experiment, seedlings belonging to two families, 'Ligol' x 'Delbard Jubile' and 'Linda' x 'Golden Delicious' were investigated. In the second experiment, two seedling populations, 'Free Redstar' x 'Melodie" and 'Free Redstar' x 'Coop 38' were tested as well. In the first experiment, nine experimental treatments were studied. They involved two growing media (peat substrate and mixture of peat substrate with compost soil 1:1), two mineral fertilizers (Osmocote Plus and Azofoska - the Polish manufactured fertilizer) and two bioregulators (Gibrescol 10 MG containing GA_3 and Ethrel). In the second experiment, additionally compost soil was included. It was found that Ethrel applied twice on young apple seedlings grown in high plastic tunnel, in the first and second year of cultivation in both experiments, shortened the juvenile stage of seedlings grafted and grown on M.9 rootstock. In the first year after planting in the selection field (fourth year after seed germination), 25% of the seedlings produced flowers, and in the second year all of them flowered and produced fruits.

Keywords: *Malus x domestica*, ethephon, breeding, selection, juvenility, fruiting

1. Introduction

The juvenile phase is the period in the life cycle of woody plants in which they are unable to produce flowers and fruits. The long juvenile stage of apple seedlings prolongs the time of conventional breeding new apple cultivars and substantially increases costs of breeding programs. In apple seedlings, the length of the juvenile stage depends on the parental genotypes used in the crossing program and may last from 5 to 12 years (Visser, 1964; Fischer, 1994). To lower the costs of apple breeding and to increase its effectiveness apple breeders conducted in the past different studies to accelerate the first flowering of apple seedlings. These involved primarily the agro-technical approaches such as promoting the intensive growth of seedlings, grafting seedlings' tops (scions) onto dwarfing rootstocks, trunk ringing, bark scoring inversion, root pruning, stem defoliation and others. A review of different agro-technical approaches to induce early flowering of apple seedlings has been given by Flachovsky et al. (2009). There are many reports in the literature indicating that application of different plant growth regulators, including 2-chloroethylphoshonic acid (Ethrel, ethephon) can affect the flower differentiation and fruiting of cultivated varieties (Luckwill, 1973; Katzfuss & Schmidt, 1986; Hamad & Mohammad, 1990). However, there are only reports on application of ethephon to promote early flower differentiation in apple seedlings. Kender (1971) reported applying ethephon as a foliar spray at different concentrations to 3, 4, and 5 year old apple seedlings in 1969 and 1970. The percentage of trees flowering for the first time in 1970 was significantly increased by application of ethephon at 1000 ppm over control. The number of flower clusters per tree was also increased by ethephon treatment over controls. In this study ethephon applied to young Spijon trees in the greenhouse resulted in an increase in the number of flower clusters produced. The author indicated that this technique may be useful in inducing early fruiting in breeding lines. Guo, Guo, and Zan (1992) also studied the effect of spraying growth retardants on the duration of the juvenile stage of apple seedlings. His results indicated that spraying with PP333 at 1000 ppm + ethephon at 1000 ppm was the most effective treatment in shortening the juvenile stage. Good treatments were also PP333 at 1500 ppm and ethephon at 1000 ppm. The aim of the studies presented in this publication was to shorten the juvenile stage of apple seedlings in the classical apple breeding program through different agro-technical approaches applied in combination with the use of plant growth regulators.

2. Methods and Materials

Investigation was based on two separate experiments: Experiment 1 - conducted between 1998 and 2005 and Experiment 2 - conducted between 2000 and 2007. Both experiments were conducted in a similar way - first two years in the high plastic tunnel and then in the open field. In both experiments apple seedlings belonging to two different hybrid families were studied. Seedlings studied in Experiment 1 were derived from the cross made between 'Ligol' x 'Delbard Jubilee' and 'Linda' x 'Golden Delicious' and in Experiment 2 seedlings were obtained from a cross made between 'Free Redstar' x 'Melodie' and 'Free Redstar' x 'Coop 38'. Following stratification, germinating seeds (February, 1999, 2001) were planted individually into small pots filled with mixture of peat substrate, compost soil and sand in proportion 1:1:1. Pots were placed in the glasshouse at 22/18^{o}C day/night temperature with artificial lighting (High-Pressure Sodium Lamps) at irradiance of 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with 16h photoperiod. When seedlings grew to the height of about 10 cm (about mid-April, 1999, 2001), they were transplanted into black plastic containers containing about 8 dm^3 of growing medium and placed into the high plastic tunnel. They were there grown on their own roots for two years. In the first experiment, nine experimental treatments were studied. They involved two growing media (commercial peat substrate and mixture of peat substrate with compost soil in proportion 1:1), two mineral fertilizers (Osmocote Plus and Polish manufactured fertilizer Azofoska) and two bioregulators (Gibrescol 10 MG containing GA_3 and Ethrel) applied in the combinations shown in Table 1. In the second experiment, similar treatments were studied but additionally compost soil was included as the growing medium (Table 2). Both fertilizers were applied at following doses directly after planting of seedlings: Osmocote Plus - 16 g/container and Azofoska – 16 g/container and additionally 8 g/container 2 month later. Gibrescol 10 MG (10% giberelic acid GA_3) was applied as foliar spray at concentration 200 mg / 1 L H_2O, 8 times in each growing season, every 15 days, from May 15th to August 31st). Ethrel 480 SL (480 g ethephon in 1litre) was applied as one foliar spray at concentration of 2000 ppm in each growing season on September 15th. Characteristics of the growing media and fertilizers used in the experiments are given in Tables 3 and 4. At the end of the second year of growing seedlings on their own roots in high plastic tunnels (December 2000 and 2002), tops of the seedlings (scions) were collected and grafted onto virus-free M.9 rootstocks in March 2001 and 2003 (winter grafting). At the beginning of April, the grafts were planted into the 8 liter black plastic containers filled with the same growing media in which seedlings were grown on their own roots. Plants were fertilized as in the two previous years but no plant growth regulators were applied. By mid-August of the same year, the grafts reached height of about 150 cm and were planted out into the open field. In both experiments each treatment comprised of 32 seedlings divided into 4 replications (blocks) with 8 plants in each replicate. The plants under high tunnel and these transplanted to the field were irrigated and fertigated using automatically controlled drip irrigation system. Results concerning the growth vigour and yielding of seedlings were statistically analyzed using the analysis of the variance technique and the means were separated by Duncan's multiple range test at P=5%.

Table 1. Height of apple seedlings (cm) on their own roots in December 1999 and December 2000 grown in high plastic tunnel (Experiment 1)

Experimental treatments	Hybrid family 'Ligol' x 'Delbard Jubile'		Hybrid family 'Linda' x 'Golden Delicious'	
	1999	2000	1999	2000
1. Peat substrate	133 e-h[z]	178 b	147 cd	204 ab
2. Peat substrate + Osmocote	147 gh	198 b-d	144 cd	232 bc
3. Peat substrate + Osmocote + Gibrescol	146 gh	212 c-f	152 d	250 c
4. Peat substrate + Osmocote + Gibrescol + Ethrel	141 e-h	206 b-f	153 d	231 bc
5. Peat substrate + Compost soil (control)	83 a	145 a	111 ab	179 a
6. Peat substrate + Compost soil + Osmocote	96 ab	187 bc	130 bc	215 b
7. Peat substrate + Compost soil + Osmocote + Gibrescol	121 c-e	220 d-f	123 ab	220 bc
8. Peat substrate + Compost soil + Osmocote + Gibrescol + Ethrel	102 a-c	178 b	105 a	214 b
9. Peat substrate + Compost soil + Azofoska + Gibrescol + Ethrel	108 b-d	201 b-e	105 a	227 bc
Average for hybrid families	119.7 a	191.7 b	130.0 a	219.1 b

[z]Means in the columns followed by the same letter do not differ significantly at P=0.05 according to Duncan's multiple range test.

Table 2. Height of apple seedlings (cm) on their own roots in December 2001 and December 2002 grown in high plastic tunnel (Experiment 2)

Experimental treatments		Hybrid family 'Free Redstar' x 'Melodie'		Hybrid family 'Free Redstar' x 'Coop 38'	
		2001	2002	2001	2002
1.	Peat substrate	135 a [z]	168 a	129 ab	229 bc
2.	Peat substrate + Osmocote	132 a	210 b	134 a-c	218 a-c
3.	Peat substrate + Osmocote + Gibrescol	135 a	227 bc	110 a	203 ab
4.	Peat substrate + Osmocote + Gibrescol + Ethrel	151 a	271 c-e	144 bc	261 de
5.	Peat substrate + Azofoska + Gibrescol + Ethrel	181 b	309 e	156 c	297 f
6.	Compost soil	131 a	290 de	140 bc	271 ef
7.	Peat substrate + compost soil (control)	131 a	260 cd	121 ab	191 a
8.	Peat substrate + compost soil + Osmocote	140 a	262 cd	125 ab	237 cd
9.	Peat substrate + compost soil + Osmocote + Gibrescol	133 a	232 bc	124 ab	230 bc
10.	Peat substrate + Compost soil+ Osmocote + Gibrescol + Ethrel	138 a	241 bc	122 ab	238 cd
11.	Peat substrate + Compost soil + Azofoska + Gibrescol + Ethrel	133 a	242 bc	110 a	204 ab
Average for hybrid families		140.0 a	246.6 b	128.6 a	234.5 b

[z]Means in the columns followed by the same letter do not differ significantly at P=0.05 according to Duncan's multiple range test.

Table 3. The pH and content of macroelements in growing media used in production of seedlings in high plastic tunnel on their own roots and grafted on M.9 in Experiment 1 and Experiment 2

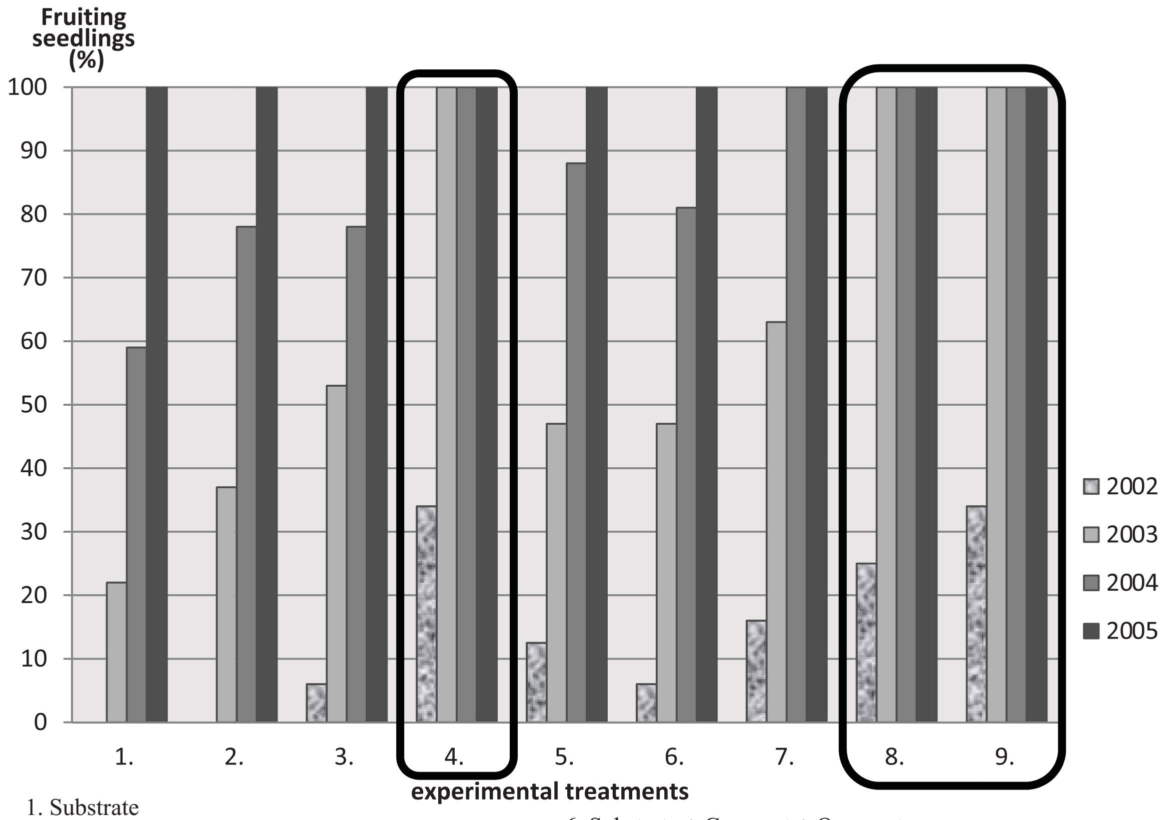
	Growing medium	pH	P	K	Mg
			mg/100 g of growing medium		
Experiment 1	Peat substrate	5.8	16.5	45.4	14.0
	Mixture of peat substrate and compost soil (1:1)	6.5	15.8	48.4	13.2
Experiment 2	Peat substrate	5.8	17.6	49.8	15.7
	Compost soil	6.6	18.2	51.6	14.9
	Mixture of peat substrate and compost soil (1:1)	6.3	16.2	50.1	14.4

Table 4. Composition of mineral fertilizers used in production of seedlings in high plastic tunnel on their own roots and grafted on M.9 in Experiment 1 and Experiment 2

	Type of fertilizer	N (%)	P_2O_5 (%)	K_2O (%)	MgO (%)	Fe (%)	Mn (%)	Cu (%)	B (%)	Mo (%)	Zn (%)
Experiment 1	Osmocote Plus 8-9	16.0	8.0	12.0	2.0	0.4	0.06	0.05	0.02	0.02	0.015
	Azofoska	13.6	6.4	19.1	4.5	0.27	0.045	0.18	0.045	0.082	0.045
Experiment 2	Osmocote Plus 5-6	15.0	10.0	12.0	2.0	0.4	0.06	0.05	0.02	0.02	0.015
	Azofoska	13.6	6.4	19.1	4.5	0.27	0.045	0.18	0.045	0.082	0.045

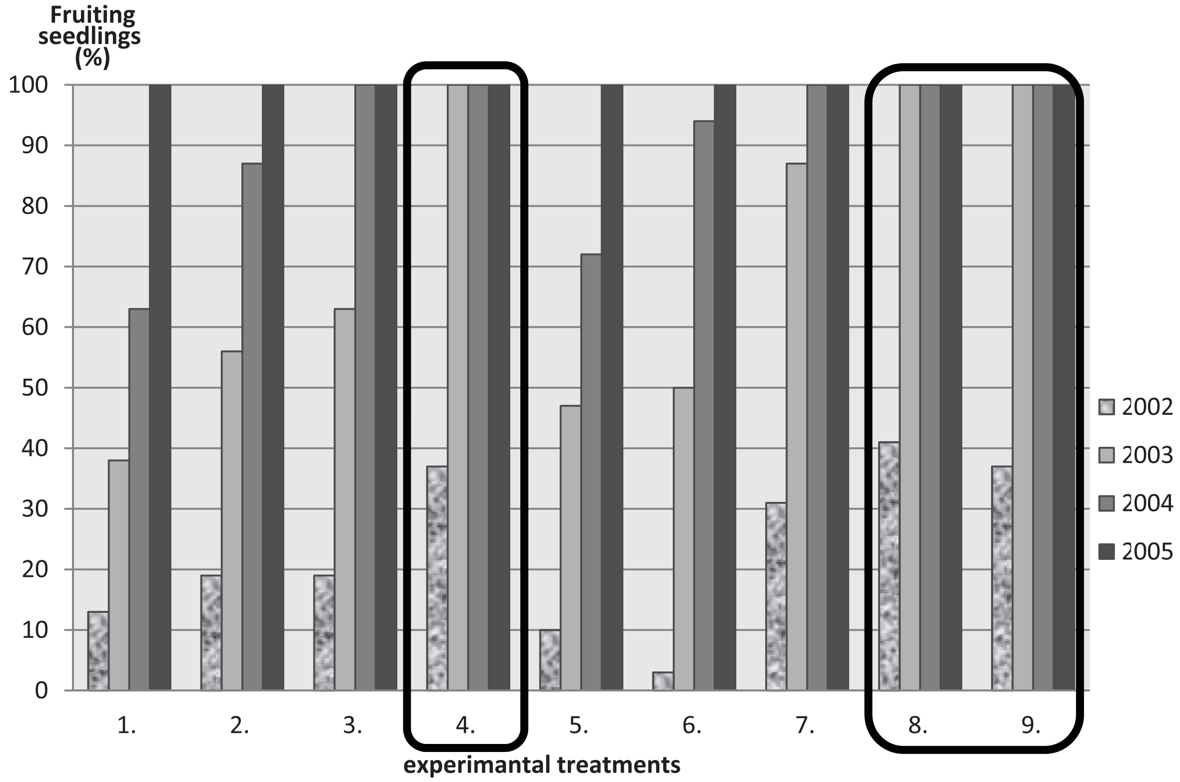
3. Results and Discussion

Results showing effects of the studied treatments on the growth of seedlings belonging to the four different apple hybrid families are presented in Tables 3 and 4, and in Figures 1-4. It can be seen from these tables that the height of seedlings in each experiment was dependent on: (i) the parental forms used for the crosses and (ii) on the experimental treatments studied. However, independently of the parentage and applied treatment combinations seedlings grown on their own roots in high plastic tunnel reached on average the height of about of 200 cm or even well exceeded this height by the end of the second year. It means that the way we cultivated our seedlings was very efficient in promoting their vigor allowing them become much taller (about 50% taller) compared to the traditional cultivation in open ground. Fischer (1994) informed that in general the part above 180 cm stem height in apple seedling is mature (adult) and can be used as scion, and directly grafted onto M.9 rootstocks. In such case, some of the trees begin to flower even three years after seeding instead of conventional 5 to 12 years. In the above studies she was producing seedlings for two consecutive years in the glasshouse supplemented with additional artificial light sources starting from January. This system was efficient in getting tall seedlings but was much more expensive than measures used for promoting growth of seedlings in our studies. Our seedlings on average were taller than 180 cm. In spite of that, not all trees (obtained from grafting of the seedlings scions onto the dwarf M.9 rootstock) flowered and produced fruits in the first year after planting into selection field (fourth year after germinating) (Figures 1–4). Depending on the parentage of seedlings and the experimental treatment applied, the percentage of flowering and fruiting seedlings varied from 0 to 41%. In each hybrid family the greatest number of flowering and fruiting seedlings was observed in those treatments where Ethrel was applied on seedlings grown on their own roots in a high plastic tunnel. The proportion (share) of the fruiting seedlings in this treatment varied from 25 to 41%. In the second year after planting of grafted seedlings into the field (fifth year after seed germinating), the number of fruiting seedling has increased. However, independently of the parentage, 100% of flowering and fruiting seedlings was observed only in the treatment where Ethrel was applied. These seedlings produced enough fruits to evaluate them for the quality traits. All of these seedlings also produced fruits in the consecutive 2 years when the experiment was conducted, while all the seedlings (100%) from other treatments produced fruits two years later. So, our results coincide with the earlier reports of Kender (1971) and Guo et al. (1992) who also informed that ethephon has a very beneficial effect on shortening the juvenile phase of apple seedlings and can promote the formation of flower buds in these seedlings much earlier than untreated controls.



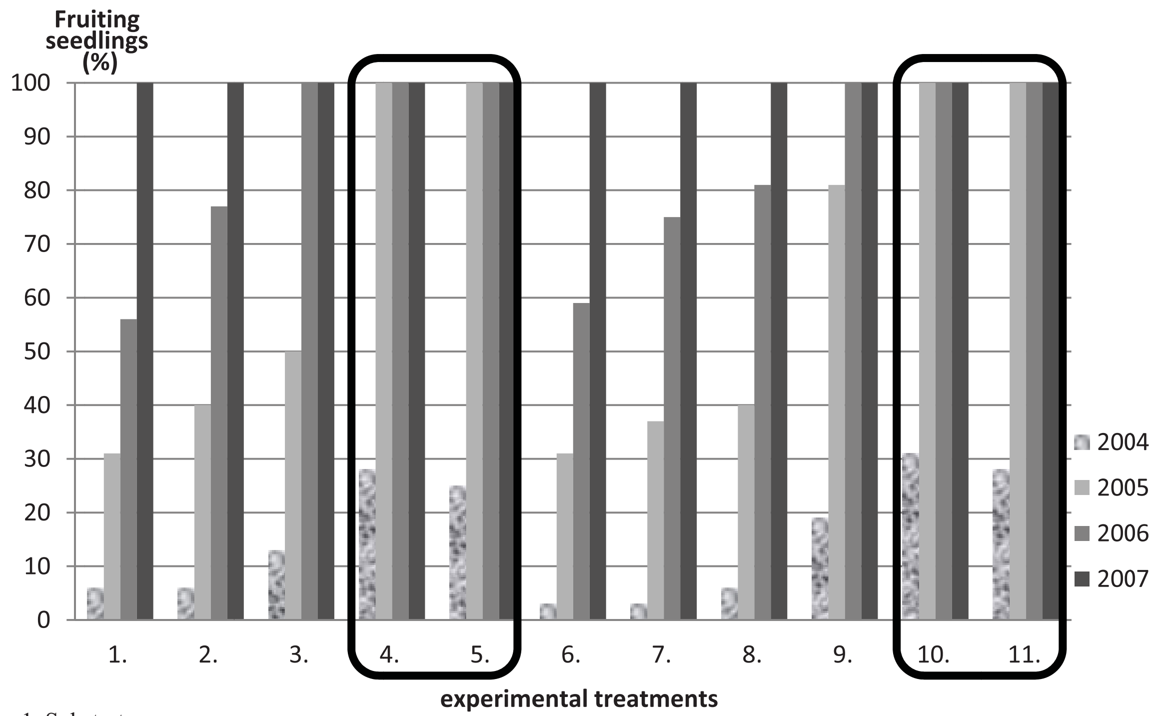
1. Substrate
2. Substrate + Osmocote
3. Substrate + Osmocote + Gibrescol
4. Substrate + Osmocote + Gibrescol + Ethrel
5. Substrate + Compost (control)
6. Substrate + Compost + Osmocote
7. Substrate + Compost + Osmocote + Gibrescol
8. Substrate + Compost + Osmocote + Gibrescol + Ethrel
9. Substrate + Compost + Azofoska + Gibrescol + Ethtrel

Figure 1. Percent of fruiting apple seedlings (‘Ligol’ x ‘Delbard Jubile’) in the field on M.9



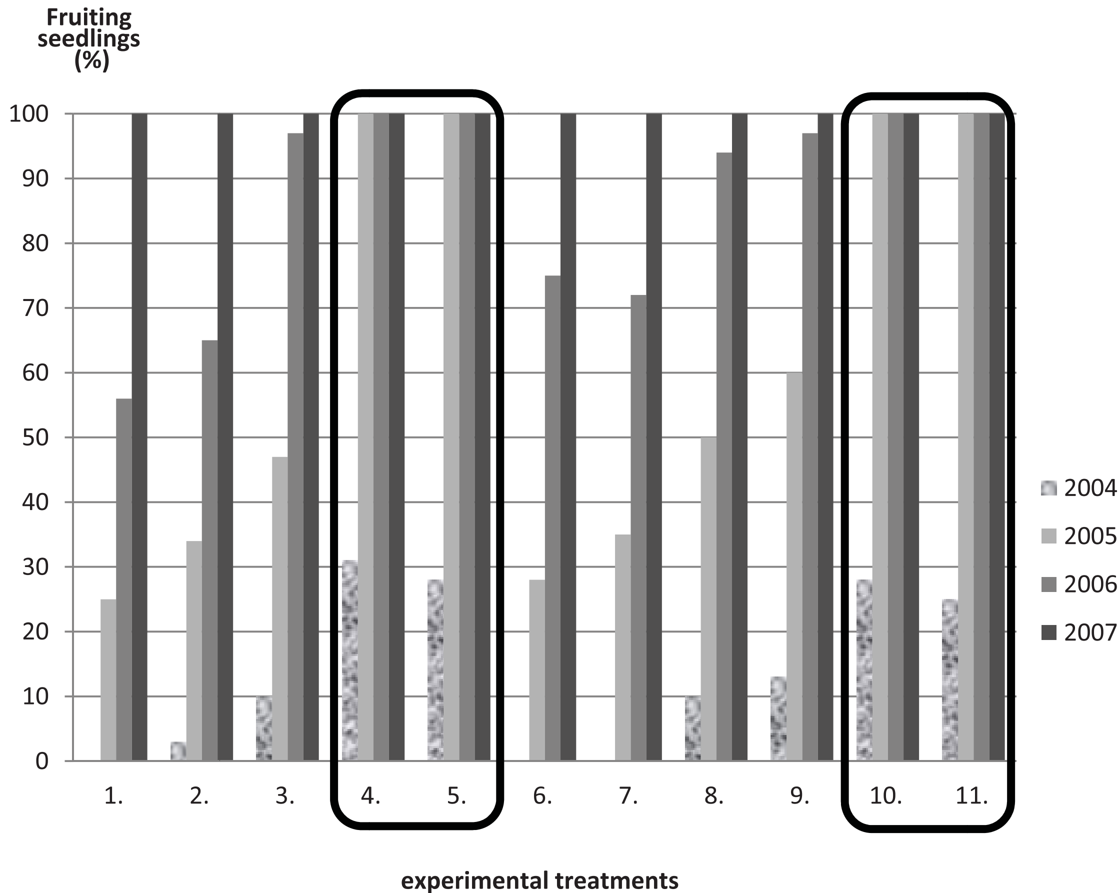
1. Substrate
2. Substrate + Osmocote
3. Substrate + Osmocote + Gibrescol
4. Substrate + Osmocote + Gibrescol + Ethrel
5. Substrate + Compost (control)
6. Substrate + Compost + Osmocote
7. Substrate + Compost + Osmocote + Gibrescol
8. Substrate + Compost + Osmocote + Gibrescol + Ethrel
9. Substrate + Compost + Azofoska + Gibrescol + Ethtrel

Figure 2. Percent of fruiting apple seedlings (‘Linda’ x ‘Golden Delicious’) in the field on M.9



1. Substrate
2. Substrate + Osmocote
3. Substrate+ Osmocote + Gibrescol
4. Substrate + Osmocote + Gibrescol + Ethrel
5. Substrate + Azofoska + Gibrescol + Ethrel
6. Compost
7. Substrate + Compost (control)
8. Substrate + Compost + Osmocote
9. Substrate + Compost + Osmocote + Gibrescol
10. Substrate + Compost + Osmocote + Gibrescol + Ethrel
11. Substrate + Compost + Azofoska + Gibrescol + Ethrel

Figure 3. Percent of fruiting apple seedlings (‘Free Redstar’ x ‘Melodie’) on M.9



1. Substrate
2. Substrate + Osmocote
3. Substrate+ Osmocote + Gibrescol
4. Substrate + Osmocote + Gibrescol + Ethrel
5. Substrate + Azofoska + Gibrescol + Ethrel
6. Compost
7. Substrate + Compost (control)
8. Substrate + Compost + Osmocote
9. Substrate + Compost + Osmocote + Gibrescol
10. Substrate + Compost + Osmocote + Gibrescol + Ethrel
11. Substrate + Compost + Azofoska + Gibrescol + Ethrel

Figure 4. Percent of fruiting apple seedlings (‘Free Redstar’ x ‘Coop 38’) on M.9

4. Conclussions

Cultivation of young apple seedlings in containers in high plastic tunnels allows breeders to produce tall seedlings in a relatively short time. At the end of the first year, most of them reach the height of more than 100 cm and at the end of the second year of cultivation most of them are at least 200 cm tall. Both growing media – peat substrate and compost soil are suitable for the cultivation of apple seedlings in containers in high plastic tunnels, however compost soil is less expensive than peat substrate. Mineral fertilizers – Osmocote and Azofoska are equally effective in promoting strong growth of apple seedlings cultivated in containers, but Azofoska is much more economical to use than Osmocote and can be a good substitute for Osmocote. Giberelic acid (GA_3) stimulates growth of apple seedlings cultivated in containers on their own roots in plastic tunnels as compared to the treatment without this bioregulator. Additional treatment of these seedlings with Ethrel does not affect their growth vigor. However, Ethrel applied on young apple seedlings cultivated in a high plastic tunnel shortens their juvenile period. In the first year after planting in the field (4th year after seed germination) flowers are present on at least 25% of the seedlings, and in the second year (5th year after seed germination) all seedlings (100%) produce fruits suitable for evaluation.

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Adaptation of the Use of Pyroligneous Acid in Control of Caterpillars and Agronomic Performance of the Soybean Crop

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Abstract

The objective of this study was to evaluate the effect of pyroligneous acid alone and in combination with insecticides in control of caterpillars and on the agronomic performance of soybean. The experiment was conducted under field and laboratory conditions in Bom Jesus - PI, in the 2010/2011 and 2011/2012 crop seasons. The field experiment used in a randomized block, 4 x 4 factorial design with four replications, the factors consisting of four pyroligneous acid concentrations (zero, 1.5, 3.0, and 6.0%) and five insecticide treatments: lambdacyhalothrin + thiamethoxam (15.9 g ha^{-1} + 21.15 g ha^{-1}), cypermethrin (300 g ha^{-1}), chlorpyriphos (240 g ha^{-1}), teflubenzuron (7.5 g ha^{-1}). In the laboratory a completely randomized design was used with 12 treatments and five replications. We evaluated the control of *A. gemmatalis* and *P. includens* (in field and laboratory), agronomic characteristics and yield. The application of pyroligneous acid alone or in combination with insecticides provided levels of control of *A. gemmatalis* and *P. includens* similar to the insecticides tested. There was no effect of pyroligneous acid on the number of pods $plant^{-1}$ or grains pod^{-1}. The yield was higher than the control, but did not differ from the other treatments containing pyroligneous acid alone or in combination. According to laboratory and field tests, it appears that the pyroligneous extract affected the number of larvae $plant^{-1}$ and reduced leaf consumption, an important result for management programs of *A. gemmatalis* and *P. includens*.

Keywords: soybean looper, soybean caterpillar, Biopirol®, grain production

1. Introdution

The Brazilian Cerrado has distinguished itself in the national scenario as a region with great potential for grain production, principally with soybean, attracting to the region numerous multinational companies in the agricultural defensives sector, as a function of the elevated demand for inputs.

The agricultural defensives sector began the year of 2012 with the expectation of 10% revenue growth, the consumption of insecticides being responsible for 21% of the total volume consumed (Sindag, 2012). The growth of the sector is driven by the traditional crops in demand, such as soybean, corn and cotton.

As a result of the difficulty of controlling caterpillars and loss of productivity due to defoliation, producers have used sequential spraying, seeking to avoid an increase in pest population density. In the meantime, there are many questions concerning the most appropriate time for spraying and the most efficient insecticides, seeking the satisfactory control mainly of caterpillars in the subfamily Plusiinae on soybeans. This fact gains distinction, seeing the importance in the national and international scenario of the soybean crop and owing to the population increase in comparison with the soybean caterpillar (*Anticarsia gemmatalis*) (Guedes et al., 2011).

The increased use of insecticides in soybean, where many times the applications are made indiscriminately, whether at high or low doses, using products often highly toxic to the environment, has led to a series of problems in the management of pests, among them the resistance of the insects to insecticides (Romero et al., 2007; Silva et al., 2011).

The evolution of pest resistance to pesticides has become one of the greatest obstacles to control programs involving the use of chemical products. The reported cases of resistance intensified with the introduction of organo-synthetic insecticides and miticides around the 1940s (Silva et al., 2011).

With the growth of insect populations resistant to insecticides, especially to the traditional chemical groups, and the interference of these with environment, research has increasingly focused on the search for new chemicals and new molecules of natural origin and less harmful to the environment. One of the products that might be an alternative to control pests in soybeans is pyroligneous acid.

Its qualities have been known for decades in Japan and were disseminated in Brazil by immigrants from that country, especially in meetings of the Association of Natural Agricultural Producers (APAN) (FAPEMIG, 2005), being used as "organic fertilizer" in rice (*Oryza sativa* L.) (Tsuzuki et al., 2000), sorghum (*Sorghum bicolor* L.) (Esechie et al., 1998) and sweet potato (*Ipomoea batatas* L.) (Shibayama et al., 1998), and as a nematicide (Cuadra et al., 2000) and fungicide (Numata et al., 1994). Recently, Petter et al. (2012) found positive effects of pyroligneous acid as a reducer of pH efficient at stabilizing spray pesticides.

Pyroligneous acid is a product obtained from the pyrolysis of wood, where condensable gases are catalyzed. This product contains about 100 chemicals, including methanol (0.1% to 1.0%), acetone (0.2%) and water (85% to 90%), with predominantly acetic acid (5.0% to 6.0%) and pH between 2.0 and 3.0 (BIOCARBO, 2001).

Not being a marketed product, little information is available in the national literature on studies of pyroligneous acid in the control of major crop pests, thus making it necessary to conduct research in order to adapt its use to generate a new technology for control of these pests, associating efficiency of control with low environmental impact.

There is reason to believe pyroligneous acid may be effective not only as an insecticide but also as a foliar fertilizer and pH reducer. Therefore, this study aimed to evaluate the effectiveness of pyroligneous acid applied alone and in combination with other insecticides in control of caterpillars and on the agronomic performance of soybean.

2. Materials and Methods

2.1 Field Experiment

Field experiment was conducted in commercial crop in Bom Jesus-PI in soil classified as dystrophic Yellow Latosol (LAd) and in the laboratory on the campus of the Federal University of Piauí (UFPI) in Bom Jesus-PI (09º 04'28"South latitude, 44° 21'31" west longitude), between October 2010 to June 2012. The field experiment involved two growing seasons (2010/2011 and 2011/2012).

The sowing of soybean (cultivar Monsoy 9350) was done on November 20, 2010 and December 10, 2011, distributing 13 seeds per meter, with spacing of 0.45 m between rows and sowing depth of 2-3 cm. N-P_2O_5-K_2O fertilizer of the formula 02-20-18 was applied at 400 kg ha^{-1}.

The experimental design was randomized block, 4 x 4 factorial, the factors consisting of four acid concentrations of pyroligneous acid (zero, 1.5, 3.0, and 6.0%) from the commercial product Biopirol®, applied alone and in combination with four insecticide treatments: lambdacyhalothrin + thiamethoxam (15.9 g ha^{-1}+ 21.15 g ha^{-1}), cypermethrin (300 g ha^{-1}), chlorpyriphos (240 g ha^{-1}), teflubenzuron (7.5 g ha^{-1}), thus totaling 16 treatments, performed in four replicates.

Each plot consisted of nine lines of crop 5 m in length, totaling 20.25 m^2; the useful area for evaluation was 12.60 m^2, once a line on each side of the main plot and 0.5 m at each end were excluded. Treatments were applied 50 days after crop emergence, using a backpack sprayer pressurized with CO_2, the rod being attached to four XR 110.020 spray nozzles, applying a spray volume equivalent to 125 L ha^{-1}.

At 7, 14 and 28 days after application (DAA) the following evaluations were made: visual crop phytotoxicity, using a percentage scale ranging from 0 to 100%, where zero means no visual symptoms and 100% the death all soybean plants; soybean caterpillar control, evaluating the number of caterpillars, using the Embrapa (2000) method of three cloth beats/plot.

At soybean harvest, the number of pods per plant and number of seeds per pod were counted, collecting from 20 plants per plot. Later, grain yield was determined with subsequent standardization of grain moisture at 14%, collecting from all plants in the active plot area.

For the field data, combined analysis of variance was evaluated for the two crop seasons, the averages of the significant variables grouped by Tukey test at 5% significance.

2.2 Laboratory Bioassays

The insect pests used in all the bioassays, *Anticarsia gemmatalis* and *Pseudoplusia includens*, were collected in the field. Adults of *A. gemmatalis* and *P. includens* were placed in PVC cages (30 cm high x 20 cm diameter) lined with bond paper serving as substrate for oviposition and maintained in a climate-controlled room (temperature: 27±2°C, relative humidity: 60±10% and photoperiod: 14 hours). Adults were fed with a cotton swab soaked in 10% honey solution, placed on a petri dish at the bottom of the cage. After the onset of oviposition, eggs were collected every 2 days. The eggs were placed in plastic cups until the emergence of caterpillars, which were transferred to artificial diet proposed by Greene et al. (1976), according to the behavior of each caterpillar.

2.2.1 *Topical Bioassay*

Topical application of pyroligneous acid was made in the third instar larvae of *A. gemmatalis* and *P. includens*. Ten µL of solution was applied to each insect on the mesothorax between the second and third pair of legs. This droplet size was chosen to ensure efficient coverage without product loss due to runoff. The pyroligneous acid was diluted with water and applied in concentrations between 0 and 500 µL/mL. After application, the larvae were placed in Petri dishes lined with filter paper containing artificial diet and kept in a temperature-controlled room (temperature: 27±2°C, relative humidity: 80±10% and photoperiod: 14 hours).

The experimental design was a completely randomized design with 12 treatments and 5 replicates, each replicate consisting of 10 insects. The evaluation of the deleterious effects of pyroligneous acid on the insects was based on the following biological parameters: mortality, duration of larval and pupal period, larval and pupal viability, and larval and pupal weight. The laboratory data were submitted to analysis of variance and the means of significant variables grouped by Tukey test at 5% significance.

2.2.2 Choice Bioassay

A. gemmatalis *and* P. includens *on soybean leaves treated with different concentrations of pyroligneous acid*: Food preference of *A. gemmatalis* and *P. includens* was evaluated on soybean leaves treated with pyroligneous acid at different concentrations. Leaves of the soybean cultivar Monsoy 9350 were obtained from plants in the V5 stage maintained in pots in a green house. The leaves were washed and immersed in a pyroligneous acid solution for about 5 minutes, subsequently removing excess moisture. Thereafter, discs were cut from along the sides of the leaves, excluding the midrib, with the aid of a punch 5 cm in diameter. The leaf area of the disks was then measured in cm², making use of a leaf area meter (Portable LI-COR model LI-3000 A).

After measurement the discs were transferred to Petri dishes containing plaster and lined with moistened filter paper. The plaster served as a base to fix the leaf discs with pins, while the moistened filter paper prevented the drying of leaf tissue. On each plate four discs were placed, a control and three concentrations of pyroligneous acid, equidistant from a central point, where a 4th instar caterpillar of *A. gemmatalis* or *P. includens* raised on artificial diet (Greene et al., 1976) was released. Five replicates were performed. The plates were covered and sealed with adhesive PVC film.

The evaluation was performed 48 hours after release of larvae, and leftover food was again measured in cm² with the help of leaf area meter. The leaf area consumed was obtained by subtracting the final area of the unconsumed discs from the starting area. Preference indices were calculated by the method of Kogan and Goeden (1970), using the formula:

$$C = 2a / (M + A),$$

where:

C = Preference index

A = consumption of test plant

M = consumption of standard plant

The interpretation of the results was in accordance with the value of C obtained, namely:

C> 1 test plant was preferred by the insect relative to the standard plant;

C = 1 plant test is similar to the standard plant in terms of preference;

C <1 test plant is less suitable than a standard plant.

The experimental design was fully randomized. The results were submitted to analysis of variance and means were compared by Tukey test at 5% significance.

3. Results and Discussion

The control of *A. gemmatalis* was significantly influenced by the treatments, with a significant interaction of the insecticide and pyroligneous acid factors in all periods (Table 1). As for the control of *P. includes*, effect was observed only at 14 days after application (DAA). With the exception of productivity, the yield components number of pods per plant and number of seeds per pod were not affected by the treatments.

Table 1. Analysis of variance (F values) combined for the effect of the different insecticides, pyroligneous acid doses and crop seasons on control of caterpillars, yield components and soybean productivity. Bom Jesus - PI, 2010/2011 and 2011/2012 crops

Sources of variance	No. Individuals *Anticarsia gemmatalis*			No. Individuals *Pseudoplusia includens*		
	7 DAA	14 DAA	28 DAA	7 DAA	14 DAA	28 DAA
Insecticides (I)	2.20^{ns}	1.57^{ns}	43.1**	0.69^{ns}	2.03^{ns}	1.37^{ns}
Pyroligneous Acid (AP)	0.27^{ns}	2.14^{ns}	34.66**	0.25^{ns}	0.74^{ns}	1.26^{ns}
Crop (S)	0.21^{ns}	0.32^{ns}	0.42^{ns}	0.06^{ns}	0.08^{ns}	0.07^{ns}
Insecticide x Pyroligneous Acid	1.76*	4.66**	34.66**	0.88^{ns}	1.84*	0.63^{ns}
Insecticide x Crops	0.03^{ns}	0.02^{ns}	1.2^{ns}	0.05^{ns}	0.02^{ns}	0.03^{ns}
Pyroligneous Acid x Crops	0.03^{ns}	0.02^{ns}	1.3^{ns}	0.03^{ns}	0.01^{ns}	0.02^{ns}
I x AP x S	0.04^{ns}	0.03^{ns}	1.5^{ns}	0.04^{ns}	0.03^{ns}	0.04^{ns}
C.V.	27.9	24.0	7.70	12.7	16.3	14.4
	No. pods plant^{-1}		No. grains pod^{-1}		Yield	
Insecticides (I)	1.41^{ns}		0.71^{ns}		0.80^{ns}	
Pyroligneous Acid (AP)	1.68^{ns}		0.21^{ns}		2.16^{ns}	
Crop (S)	0.41^{ns}		0.32^{ns}		0.23^{ns}	
Insecticide x Pyroligneous Acid	0.50^{ns}		0.28^{ns}		2.39*	
Insecticide x Crops	0.32^{ns}		0.30^{ns}		0.24^{ns}	
Pyroligneous Acid x Crops	0.25^{ns}		0.41^{ns}		0.34^{ns}	
I x AP x S	0.31^{ns}		0.32^{ns}		0.45^{ns}	
C.V.	15.4		21.8		13.0	

* e ** significant at 5 and 1 % respectively; ^{ns} - not significant. DAA - Days after application of treatments. C.V. – Coefficient of variation. Crop seasons: 2010/2011 and 2011/2012.

At 7 DAA only the treatment composed of the mixture of cypermethrin and 6% pyroligneous acid presented lesser control of *A. gemmatalis* (Table 2). Already at 14 and 28 DAA, with the exception of the without insecticide treatment and in the absence of pyroligneous acid that had the worst control, the other treatments did not differ statistically among themselves, thus proving the efficiency of insecticides and pyroligneous acid in the control of *A. gemmatalis*. It is interesting to note the residual effect of pyroligneous acid over the 28 days of evaluation, which kept the infestation within the level of economic injury for this pest. These data differ from those found by Bogorni et al. (2008), who found no effect of the pyroligneous extract in control of *Tuta absoluta* (tomato pinworm). Importantly, the cited work was developed in the laboratory, and under field conditions the data could differ, as is the case in this work.

Table 2. Number of individuals of *Anticarsia gemmatalis* from three cloth beats, after applying combinations of insecticides and pyroligneous acid concentrations. Bom Jesus - PI, 2010/2011 and 2011/2012 crops

Insecticides	Pyroligneous Acid (%)				Average
	0	1.5	3.0	6.0	
	7 DAA*				
lambdacyhalothrin + thiamethoxam	6 aA	4 aA	4 aA	2 aA	4
cypermethrin	4 aA	5 aA	5 aA	8 aB	6
chlorpyriphos	3 aA	6 aA	5 aA	1 aA	4
teflubenzuron	3 aA	3 aA	2 aA	4 aAB	3
without insecticide	6 aA	4 aA	2 aA	6 aAB	4
Average	4	4	4	4	4
	14 DAA				
lambdacyhalothrin + thiamethoxam	0 aA	1 aA	1 aA	1 aA	1
cypermethrin	1 aA	1 aA	0 aA	0 aA	1
chlorpyriphos	1 aA	1 aA	1 aA	0 aA	1
teflubenzuron	0 aA	0 aA	0 aA	0 aA	0
without insecticide	4 bB	1 aA	1 aA	1 aA	2
Average	1	1	1	1	1
	28 DAA				
lambdacyhalothrin + thiamethoxam	0 aA	0 aA	0 aA	0 aA	0
cypermethrin	0 aA	0 aA	0 aA	0 aA	0
chlorpyriphos	0 aA	0 aA	0 aA	0 aA	0
teflubenzuron	0 aA	0 aA	0 aA	0 aA	0
without insecticide	5 bB	0 aA	0 aA	0 aA	1
Average	1	0	0	0	1

Averages followed by the same small letters in the line (horizontal) and large letters in the column (vertical) did not differ statistically by Tukey test at 5% probability. *Days after application.

As reported by Tsuzuki et al. (2000), under field conditions the application of pyroligneous acid can activate substances of plant secondary metabolism, inducing resistance to pests. Therefore, the effect of pyroligneous acid in the control of *A. gemmatalis* may be linked to the synthesis of metabolites involved in the mechanism of plant defense, since, in addition to the pyroligneous extract presenting in its composition phenolic compounds, it presents in larger part as a constituent acetic acid, which is part of the mevalonic acid biosynthetic pathway, a precursor in the synthesis of terpenes, which are essential metabolites in the defense mechanisms of plants against attack by herbivorous insects. Campos et al. (2005) found greater accumulation of phenolic compounds after application of pyroligneous acid. It is important to emphasize also that terpenes in the form of monomers, called pyrethroids, have insecticidal activity.

For the control of *P. includens* treatment effect was seen only at 14 DAA; cypermethrin was the treatment that showed the lowest rates of control (Table 3). These results differ from those of Morandi Filho et al. (2006), who found no effect of pyroligneous extract on *Argyrotaenia sphaleropa* in the laboratory, but corroborate those of Azevedo et al. (2005), who reported on the effect of field application of pyroligneous acid in control of *Bemisia tabaci*.

Table 3. Number of individuals of *Pseudoplusia includens*, from three beats of cloth, after applying combinations of insecticides and pyroligneous acid concentrations. Bom Jesus - PI, 2010/2011 and 2011/2012 crops

Insecticides	Pyroligneous Acid (%)				Average
	0	1,5	3,0	6,0	
	7 DAA*				
lambdacyhalothrin + thiamethoxam	31	43	42	40	39^{ns}
cypermethrin	34	40	40	36	37
chlorpyriphos	32	35	35	39	35
teflubenzuron	47	41	34	43	41
without insecticide	43	40	41	34	40
Average	37	40	38	38	38
	14 DAA				
lambdacyhalothrin + thiamethoxam	11 aA	8 aA	12 aA	16 aA	12
cypermethrin	13 abA	20 bB	16 abA	10 aA	15
chlorpyriphos	14 aA	17 aAB	17 aA	14 aA	16
teflubenzuron	9 aA	12 aAB	17 aA	11 aA	12
without insecticide	20 bA	1 aAB	12 aA	12 aA	14
Average	14	14	15	13	14
	28 DAA				
lambdacyhalothrin + thiamethoxam	10	9	9	6	8^{ns}
cypermethrin	10	9	10	10	10
chlorpyriphos	12	10	9	11	11
teflubenzuron	9	9	8	10	9
without insecticide	13	8	8	8	9
Average	11	9	9	9	9

Averages followed by the same small letters in the line (horizontal) and large letters in the column (vertical) did not differ statistically by Tukey test at 5% probability. *Days after application.

It is observed that generally all treatments showed reduced numbers of insects during the trial period. This may be linked to climatic conditions during the experiment, such as the above average rainfall. These conditions led to lower pressure from *P. includens* in the experimental area.

It can be seen that the systemically-acting insecticides in combination with pyroligneous acid showed better control of *A. gemmatalis* and *P. includens*, which may also be associated with greater absorption of the products, because of a possible coordination complex effect of the acid (formation of chelates) with the insecticide molecules, by electrochemical bonds with the carboxyl and phenolic groups in the extract, which in turn have greater facility of penetration via the cuticle and plasma membrane. According to Zanetti et al. (2004), there is evidence that the pyroligneous extract, due to some physico-chemical characteristics, among them the chelating effect, could enhance the efficiency of pesticides in foliar sprays.

In general, smaller yields were observed in the control and cypermethrin treatment associated with 1.5% pyroligneous acid (Table 4). This may be associated with loss of leaf area caused by the greater number of individuals of *A. gemmatalis* and *P. includens*, reducing the photosynthetic active area and hence the production of photoassimilates that would be directed to the grain. It is interesting to note that productivity in the absence of pesticides and in the presence of acid pyroligneous was unaffected, demonstrating it to be an alternative to control these pests when applied alone or in combinations with insecticides.

Table 4. Yield of soybeans after application of combinations of insecticides and pyroligneous acid concentrations. Bom Jesus - PI, 2010/2011 and 2011/2012 crops

Insecticides	Pyroligneous Acid (%)				Average
	0	1.5	3.0	6.0	
	7 DAA*				
lambdacyhalothrin + thiamethoxam	3.388 aA	4.099 aAB	3.827 aA	3.925 aA	3.809
cypermethrin	3.544 aA	3.291 aB	3.688 aA	3.902 aA	3.606
chlorpyriphos	3.555 aA	3.761 aAB	3.402 aA	3.419 aA	3.534
teflubenzuron	3.847 abA	4.311 aA	3.363 bA	4.013 abA	3.883
without insecticide	3.055 aB	3.847 aAB	3.958 aA	3.519 aA	3.594
Average	3.477	3.861	3.647	3.755	3.685

Averages followed by the same small letters in the line (horizontal) and large letters in the column (vertical) did not differ statistically by Tukey test at 5% probability. *Days after application.

There was no correlation between the components of production, number of pods $plant^{-1}$ and number of grains pod^{-1}, with productivity. Studies show that variables such as number of pods $plant^{-1}$ and number of grains pod^{-1} may not correlate with production (Stone & Moreira, 2000; Oliveira et al., 2002), due to the unevenness in the stand as well as to production components such as radiation, that are difficult to measure, leading to high coefficients of variation.

The values obtained for the duration of the larval stage of *A. gemmatalis* treated topically with different concentrations of pyroligneous acid did not differ significantly (Table 5). The data on the average duration of the larval phase were similar to those reported by Gazzoni & Tutida (1996), who obtained 13.7 days, and Machado et al. (1999), who obtained 14.8 days. In bioassays with *P. includens*, the larval period increased with increasing concentrations of pyroligneous acid. There was an adverse effect on the biology of the insect that required a greater number of days to complete the larval stage. The pupal period for both *A. gemmatalis* as for *P. includens* did not differ from control. The weight of the larvae of *A. gemmatalis* was lower at higher concentrations of pyroligneous acid, which did not affect the duration of the larval period, but affected the metabolic development because it reduced the weight of pupae of *A. gemmatalis* and *P. includens* (Table 5), which is directly related to the biotic potential. In topical application, concentrations exceeding 200 µL/mL were associated with mortality before the insects completed the cycle, thus no data are displayed in the table.

Table 5. Average duration (days), weight (mg) and viability (%) of the larval and pupal phases of *Anticarsia gemmatalis* and *Pseudoplusia includens* submitted to topical treatment with pyroligneous acid. Temperature: 27±2 ºC, relative humidity: 60±10%, photophase: 14 h

	Duration of Larval Period (Days)		Duration of Pupal Period (Days)		Larval Weight (mg)[2]		Pupal Weight (mg)[2]		Larval Viability (%)		Pupal Viability (%)	
	Anticarsia gemmatalis	*Pseudoplusia includens*	*Anticarsia gemmatalis*	*Pseudoplusia includens*	*Anticarsia gemmatalis*	*Pseudoplusia includens*	*Anticarsia gemmatalis*	*Pseudoplusia includens*	*Anticarsia gemmatalis*	*Pseudoplusia includens*	*Anticarsia gemmatalis*	*Pseudoplusia includens*
Source of Variation	Mean squares											
Treatments	7.713**	11.333**	0.54ns	0.853ns	7606.64**	0.00070ns	8724.23**	0.00893**	2051.33**	4382**	1427.12**	3040.244**
error	0.8666	1.8667	0.90	0.9667	730.73	0.000342	113.28333	0.0006898	118.33333	190	228.67961	399.59952
CV%	6.9301	7.6612	12.32	13.0512	10.7783	26.84700	7.671887	16.13931	13.65453	19.9769	21.18394	32.64577
Treatments µL/mL	Averages[1]											
200	14.2a	19.6a	7.8a	7.8a	206.8b	0.049a	123.2b	0.122b	52c	32c	41.6 b	18.7b
120	15.0a	19.2a	8.2a	8.2a	227.0b	0.066a	111.4b	0.138b	60c	40c	65.1a	48.7a
70	14.4a	18.6ab	7.8a	7.2a	216.4b	0.064a	107.2b	0.150b	78b	60b	74.2a	60.6a
50	12.2a	17.0bc	7.6a	7.6a	287.8a	0.075a	106.4b	0.140b	88ab	82a	73.4a	75.4a
10	12.6a	16.8bc	7.6a	7.2a	298.0a	0.080a	192.2a	0.204a	100a	100a	86.0a	80.0a
0	12.2a	15.8c	7.2a	7.2a	268.8a	0.079a	192.0a	0.222a	100a	100a	88.0a	84.0a

[1]Averages followed by the same letter in the columns do not different among themselves by Tukey test at 5%.
[2] Transformed data in √ x +0.5.

The viability of the larval stage for the six treatments was significantly reduced with increasing concentrations. It was observed that the caterpillars had a small body size in relation to days of life, although no abnormalities were observed. The number to instars was similar for all treatments, with five instars for both *A. gemmatalis* and *P. includens* (Table 5).

In the multiple choice test, treatment of soybean leaves with different concentrations of pyroligneous acid reduced consumption of leaf area over 48 hours, resulting in a lower preference index and explaining the data collected in the field trials, where the pyroligneous extract alone or in combination reduced the number of larvae during the trial period. At concentrations above 260 mL/ml there was no consumption as a function of larval mortality. Thus it can be inferred that pyroligneous extract has a contact and ingestion effect.

Table 6. Consumed leaf area (cm^2) and Preference Index for 4^{th} instar larvae of *Anticarsia gemmatalis* and *Pseudoplusia includens* within 48 hours in free choice test of soybean leaves treated with different concentrations of pyroligneous acid. Temperature: 27±2 ºC, RH 60±10%, photophase: 14 h

	Foliar Consumption cm^2		Preference Index	
	Anticarsia gemmatalis	*Pseudoplusia includens*	*Anticarsia gemmatalis*	*Pseudoplusia includens*
Source of Variation	Mean squares			
Treatments	8.035	2.9088	2.908	0.4510
error	0.2365	0.132	0.132	0.0064
CV%	20.860	42.908	42.908	17.811
Treatments µL/mL	Averages[1]			
0	4.12a	2.46a	1a	1a
10	3.28b	1.32b	0.88ab	0.69b
50	3.09b	0.88bc	0.85ab	0.52c
70	2.76b	0.80bc	0.79b	0.49c
120	2.01c	0.6cd	0.64c	0.42c
200	1.76c	0.30cd	0.59c	0.21d
260	1.60c	0.21cd	0.55c	0.15d
300	0d	0.13d	0d	0.09d

[1] Averages followed by different letters in the column differ significantly among themselves by Tukey test at 5%.

The literature regarding the use and efficiency of pyroligneous extract as insecticide is still limited. Thuller et al. (2007) report larval mortality for *Plutella xyslostella*, Biopirol was effective at control under the conditions tested. Silva et al. (2005, 2006) found a toxic effect of pyroligneous extract on *Atta sexdens rubropilosa*, noting that pyroligneous extract was associated with ant mortality lasting up to five days after application and eucalyptus seedlings treated by spraying and immersion in pyroligneous extract between 0.1% and 0.2% inhibited foraging activity of *Atta sexdens rubropilosa*. Also Azevedo et al. (2005) reported that pyroligneous extract increases the effectiveness of nymph control throughout plant development and reduces the effect in the control of adults at the end of the cycle. Etoffel Efron et al. (2011a) found that pyroligneous acid under the conditions tested did not have a negative effect on the survival of adult *Cryptolaemus montrouzieri*, demonstrating that the products are selective for this species. Also Etoffel Efron et al. (2011b) did not identify insecticidal activity of pyroligneous extract on *Anastrepha fraterculus* under the conditions tested. Mendonça et al. (2006), in investigating the effect of pyroligneous acid on *Leocoptera coffeella*, noted there is no negative effect on the predatory wasp of the pest. According to Peter et al. (2012) pyroligneous extract was not effective at controlling white spot in maize.

Miyasaka, Ohkawara and Utsumi (1999) found that pyroligneous extract mixed with extracts of garlic, mucuna pepper and neem is effective in controlling pests, when diluted and sprayed on the shoots of plants, making them more vigorous and improving product quality, consistent with the finding of this investigation. In both field and laboratory trials, the pyroligneous extract negatively affected the development of *A. gemmatalis* and *P. includens* and did not reduce soybean yield in the absence of insecticide and presence of pyroligneous extract.

The use of chemical products to control pests is a relevant issue since it has implications for the cost of agricultural production, rural worker safety and environmental risks. Investigations regarding the effectiveness of products in pest control, in preliminary laboratory tests or in field trials are very important. They should be performed and discussed constantly in order to avoid control failures, financial losses and negative impact on the system.

This present study addresses with initial field and laboratory testing of the use of pyroligneous extract for control of major agricultural pests of soybean, obtaining positive results in the control of *A. gemmatalis* and *P. includens*. However, it is necessary to continue the investigations as to the dose response curves, impact on beneficial organisms, soil residual effect and mechanism of action.

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Amplification and Molecular Characterization of DREB1A Transcription Factor Fragment From Finger Millet [(*Eleusine coracana* (L.) Gaertn]

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Abstract

Studies have shown that several plant species posses DREB1A and DREB2A (Dehydration-Responsive Element Binding Protein) orthologs. DREB transcription factors, also called C-repeat binding factors (CBFs), are the transacting elements/ transcription factors first identified in Arabidopsis which bind to low-temperature and dehydration responsive element (LTRE/DRE) found in several dehydrin (*Dhn*) promoters as well as in promoters of other cold and drought responsive genes and involved in dehydration-, cold-, and salinity-regulated gene expression. In this study, a fragment of *DREB1A* ortholog named *EcDREB1A* has been amplified from finger millet (*Eleusine coracana*), an important drought-tolerant grain crop with a rich genetic diversity grown in semi-arid tropics. In the current study, a systematic approach has been taken to predict a theoretical primer for *EcDREB1A* based on the cloned *Arabidopsis thaliana AtDREB1A* and other DREB genes for orthologous gene identification from finger millet. Sixteen different but related nucleotide sequences based on *AtDREB1A* gene were retrieved from different databases. A highly conserved region of 287 bp was detected on multiple sequence alignments through claustalw2 program and a set of primers (forward and reverse) was predicted using Primer3plus and Net software on the basis of this conserved region, assuming ideal conditions for primer length, GC content, formation of primer-dimers, hairpin-loops etc. The amplified genomic fragment of *EcDREB1A* was found to be 536 bp long, with possible introns as per translational analysis. Longest detected ORF in the amplified *EcDREB1A* fragment encodes a putative protein of 84 amino acids rich in serine (13.10%) with a predicted molecular mass of 9.29 kDa. Multiple sequence alignment of this *EcDREB1A* fragment with other *DREB* genes revealed presence of 9 highly conserved amino acids. Allele mining of *EcDREB1A* gene fragment across selected 5 finger millet cultivars revealed no variations on nucleotide, probably due to narrow genetic base in the test materials. Identification of novel regulatory genes involved in abiotic stress tolerance and allele mining in a manner similar to this presented herein might lead to a better and quicker solution for improving stress tolerance in crop plants.

Keywords: multiple sequence alignment, abiotic stress, PCR, DNA sequencing.

1. Introduction

Sustainable agricultural productivity requires development of crop plants tolerant to several abiotic stresses and able to grow in adverse environments. Abiotic stresses such as drought, low or high temperature, and salinity have detrimental effects on plant growth and reduce crop yield drastically. The ever increasing pressure put on agricultural land by burgeoning human populations and various human activities like mining, intense agricultural activity to increase crop productivity, the rising land occupation and the heavy and growing industrial activity have resulted in land degradation, drought and salinity across large tracts of agricultural land. Worldwide, it has been estimated that approximately 70% of yield reduction is the direct result of abiotic stresses (Acquaah, 2007). Abiotic stresses are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell (Zhu, 2001) and may cause denaturation of functional and structural proteins (Smirnoff, 1998). As a consequence, these diverse environmental stresses often activate similar cell signaling pathways (Knight, 2000; Shinozaki & Yamaguchi-Shinozaki, 1996; Zhu, 2001) and cellular responses, such as the production of stress proteins, up-regulation of antioxidants, and accumulation of compatible solutes (Zhu et al., 1997; Cushman & Bohnert, 2000). Most metabolic pathways in plants are subjected to transcriptional regulation, which in turn are often regulated through developmental, environmental, or hormonal processes. Transcription factors modulate the expression of specific groups of genes through sequence-specific DNA binding and protein-protein interaction. They can act as activators or repressors of gene expression, leading to specific effect of genes in terms of cellular response (Latchman, 2003). DREB transcription factors (Dehydration Responsive Element Binding proteins), also called C-repeat binding factors (CBFs), are the transacting elements/ transcription factors first identified in *Arabidopsis thaliana*; they bind to low-temperature and dehydration responsive element (LTRE/DRE) found in several dehydrin (*Dhn*) promoters as well as in promoters of other cold and drought responsive genes and involved in dehydration-, cold-, and salinity-regulated gene expression (Yamaguchi-Shinozaki & Shinozaki, 1994; Stockinger et al., 1997; Liu et al., 1998). DREB transcription factors (genes of *DREB* family) have been grouped into DREBl/CBF and DREB2. DREB1 includes 3 additional novel genes and comprises of DREB1A (CBF3), DREB1B (CBF1), DREB1C (CBF2), and DREB1D (CBF4). DREB2 includes 6 genes (Sakuma et al., 2002). DREB1A and DREB1B share 86% identity; DREB1B and DREB1C have 86% identity; DREB1A and DREB1C, have 87% identity (Liu, et al., 1998). DREB1D (CBF4) gene expression, however, is up-regulated by drought stress , but not by low temperature (Haake et al., 2002).

The DNA sequence information obtained from the model plant *A. thaliana* and several other species has been used towards the identification, structural and functional characterization of genes. In particular, gene sequences from the model plant *A. thaliana* offer a unique opportunity to clone cognate genes and genes from heterologous sources, resulting in discovery of new genes, allele-mining, and large-scale SNP genotyping (Yong-Li et al., 2002; Kirankumar et al., 2001). The Polymerase Chain Reaction (PCR) has proven to be a versatile tool in molecular biology. The use of this technique has generated unprecedented advances in gene discovery and gene expression analysis (Katherine et al., 2008). Optimal primer sequence and appropriate primer concentration are essential for efficiency of the PCR, hence, there is a need of systematic procedure to guide the primer design approach (Binas, 2000).

Finger millet (*Eleusine coracana* (L.) Gaertn; Ragi) is an annual plant widely grown as millet in the arid areas of Africa and Asia. As one of the drought-tolerant grain crops with a rich genetic diversity finger millet poses an excellent model crop of choice for studying the genetic and physiological mechanisms of drought tolerance. It was proposed to isolate dehydration responsive element binding factor from this plant as an variant to be useful in allele mining.

2. Method

2.1 Plant Material

Five finger millet cultivars (1. Dibyasinha, 2. Suvra, 3. Neelachal, 4. Bhairabi, and 5. Chilka) were collected from the department of Plant Breeding & Genetics, College of Agriculture, Bhubaneswar, India for the present investigation.

2.2 Isolation of Genomic DNA

The genomic DNA was isolated from seedlings (3 wks old) of fingermillet cultivars by modified CTAB method (Sambrook et al., 1989). Extraction buffer (100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl and 2% CTAB (w/v) was used for DNA extraction followed by chloroform: isoamyl alcohol extraction. DNA was precipitated by adding two volumes of cold isopropanol and was stored at -20°C overnight. It was then centrifuged for 10 min at 10,000 rpm. The supernatant was decanted and DNA pellet was washed twice with 70 per cent alcohol and air-dried. The genomic DNA was resuspended in 50µl of T10E1 buffer (Tris 10 mM and EDTA 1.0 mM). The quality and quantity of the DNA was checked spectrophotometrically and was taken for PCR reaction. The DNA was diluted to 1:100 (v/v) with T10E1 and subjected to spectrophotometer using T10E1 buffer as blank. Absorbance at 260 nm and 280 nm was recorded for checking the quality and contamination from protein and RNA. DNA concentration [µg/ml] of original sample was calculated by following the formula: Concentration of DNA [µg/ml] = OD at 260 × 50 × dilution factor.

2.3 In Silico Designing of EcDREB1A Primers

Nucleotide sequences of *DREB1A* were searched in NCBI (http://www.ncbi.nlm.nih.gov/) through Entrez for *A. thaliana* group. From the hits, 13 similar sequences were chosen including four mRNAs, five ESTs, three complete CDS, and one clone DNA. Furthermore, three genomic DNA sequences were taken from EMBL-EBI database through European Nucleotide Archive (http://www.ebi.ac.uk/ena/) interface. The information related to those sequences, including accession number, sequence type, and sequence length were reported in Table 1. In total, 16 nucleotide sequences belonging to *A. thaliana DREB1A* group were subjected to Multiple Sequence Alignment (MSA) using Clustalw2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2) of European Bioinformatics Institute, Cambridge, United Kingdom (EBI). The conserved region obtained from the MSA of 16 nucleotide sequences was used as input for designing primer using Primer3 Plus tool (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The parameters were adjusted as follows: Melting temperature: $T_{m\,min}$=52ºC, $T_{m\,max}$=58ºC ; Primer size: Min=18 bp, Max=24 bp; GC% contents: Min=50%, Max=60%. The predicted primers were subjected to check various properties; namely, hairpin loops, primer dimers, T_m and GC% using Premier Biosoft's NetPrimer tool (http://www.premierbiosoft.com/netprimer /index.html). Multiple sequence alignment was performed between the selected forward primers along with the 16 nucleotide sequences of *DREB1A* gene homologs belonging to *A. thaliana* group, using ClustalX 2.1 (Thompson et al., 1997; http://www.clustal.org). Again, those 16 nucleotide sequences were subjected for designing reverse primer using GeneFisher2 (http://bibiserv.techfak.unibielefeld.de/genefisher2/submission.html) followed by selection of the most suitable primer using Premier Biosoft's NetPrimer tool. The parameters for primer prediction were adjusted as mentioned above. Multiple sequence alignment was performed between the selected reverse primers along with the 16 nucleotide sequences of *DREB1A* gene homologs belonging to *A. thaliana* group using ClustalX 2.1. The specificity of both forward and reverse primers was checked using Blast program of NCBI database.

2.4 Orthologous Gene Amplification by PCR

Homology-based gene amplification was carried out by PCR using a set of gene-specific primers synthesized by Chromous Biotech, Bangalore. PCR amplification of *EcDREB1A* was optimized by standardizing the annealing temperature and primer concentrations using PCR (Perkin Elmer, ABI system). DNA polymerase, assay buffer, and dNTPs were purchased from Geneaid Biotech Ltd., New Taipei City, 22180 Taiwan (www.geneaid.com). The optimal PCR conditions used for amplification of *EcDREB1A* comprised 45 cycles, one for initial denaturation at 94 ºC for 5 min and 44 cycles of denaturation at 94ºC for 1 min, annealing at 52ºC for 1 min, pre- extension at 72ºC for 2 min, with the final extension at 72ºC for 10 min. The amplification was carried out with gDNA-1µl (50 ng), Forward Primer-2.4 µM, Reverse Primer-2.4 µM, dNTPs-0.4 mM, 10X *Taq* DNA Polymerase Assay Buffer-2.5 µl, *Taq*DNA Polymerase enzyme (2 units/µl)-1.0 µl and water added to make up the total reaction volume: 25 µl.

Table 1. Information regarding 16 *DREB1A* nucleotide sequences belonging to *A. thaliana* group, datamined in NCBI/ Entrez

Sl. No.	Accession No.	Sequence Type	Sequence Length
1	NM_118680.1	mRNA	908
2	AB007787.1	mRNA	908
3	AF074602.1	mRNA	902
4	BT024594.1	mRNA	651
5	CB259241.1	ESTs	613
6	CK119063.1	ESTs	748
7	DR750881.1	ESTs	757
8	DR357280.1	ESTs	425
9	DR357281.1	ESTs	493
10	AY691904.1	Complete cds	805
11	DQ372533.1	Complete cds	651
12	FJ169300.1	Complete cds	651
13	AM992886.1	Clone DNA	651
14	BAA33434.1	Genomic DNA	651
15	AAU93686	Genomic DNA	651
16	AEE85065	Genomic DNA	651

2.5 Electrophoresis and Elution of the Amplified EcDREB1A Fragment

After completion of the thermoprofile, electrophoresis was carried out to separate the amplified products in 1.5% agarose (Himedia Laboratories Pvt. Ltd., Mumbai, India) gel along with 100 bp DNA ladder (Chromous Biotech Pvt. Ltd, Bangalore, India). Large-scale amplification was performed towards elution of the PCR fragment. The single bright amplicon of appx. 0.5 Kbp was eluted using Gel/PCR DNA fragments extraction Kit (Geneaid) following the published protocol (Vogelsteinand & Gillespie, 1979).

2.6 Sequencing of Putative EcDREB1A Gene

The PCR-amplified DNA fragment of ~0.5 Kbp was sequenced by Chromous Biotech, Bangalore, India, following Applied Biosystems fluorescence-based cycle sequencing system (ABI 3500 XL Genetic Analyzer), which is an extension and refinement of Sanger's dideoxy sequencing (Sanger et al., 1974). The sequencing mix composition of 10 µl total contained: 4 µl Ready Reaction Mix, 1 µl (10 ng) Template, 2 µl (10 pmol) Primer, and 3 µl MilliQ Water; PCR Conditions used (Initial Denaturation: 96 ºC for 1 min, and 24 cycles of Denaturation: 96ºC for 10 sec, Hybridization: 50ºC for 5 sec, Elongation: 60ºC for 4 min) were followed for sequencing, using the Big Dye Terminator version 3.1" Cycle sequencing kit and Polymer & Capillary Array (POP_7 polymer 50 cm Capillary Array, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA, USA, 94404) and Applied Biosystem Micro Amp Optical 96-Well Reaction plate (Applied Biosystems, USA). Data analysis was carried out using SeqScape_ v 5.2 software (Applied Biosystem) following BDTv3-KB-Denovo_v 5.2 analysis protocol (Applied Biosystem).

2.7 Structural and Phylogenetic Analysis EcDREB1A Genomic Sequence Fragment

The PCR amplicon sequence was subjected for structural analysis. Homology search was done using BLAST algorithm available at http://www.ncbi.nim.nih.gov through Entrez for *A. thaliana* group, *Oryza sativa* Indica group, *Capsella bursa-pastoris*, *Medicago truncatula*, Rosa hybrid cultivar, *Sorghum bicolor*, and *Nicotiana tabacum*. From the hits, nine sequences including four mRNAs, two complete CDS, one clone DNA, and one genomic DNA sequence were taken from EMBL-EBI database through European Nucleotide Archive (http://www.ebi.ac.uk/ena/) interface and were subjected to MSA using Clustalw2 program (http://www.ebi.ac.uk/Tools/msa /clustalw2) of European Bioinformatics Institute (EBI, UK). The alignment parameters were kept default. Multiple sequence alignments were generated with ClustalW at the EBI ClustalW server (http://www.ebi.ac.uk/clustalw/) using default parameters (Thompson et al. 1994). The alignments were visualized using the BioEdit program (version 5.0.9 http://www.mbio.ncsu.edu/BioEdit/bioedit.html). MEGA 3.1 software was used for phylogenetic analysis [version 3.1; http://www.megasoftware.net] (Kumar et al., 2004). The phylogenetic tree was constructed using the neighbor-joining method with bootstrap test.

In silico translation of the obtained genomic DNA sequence of *EcDREB1A* was done using GENSCAN software (genes.mit.edu/GENSCANinfo.html). General features of the predicted protein (molecular weight, Iso-electric point-pI, amino acid composition) were assessed using the Prot Param tool (http://expasy.org/cgi-bin/protparam). All other bioinformatics analyses, such as homology searches etc., were performed using tools that are accessible via different links on the proteomics service site of the Swiss Institute of Bioinformatics (http://www.isb-sib.ch/forms.htm).

3. Results

3.1 In Silico Design of DREB1A Primers

A highly conserved region (100% identity) of 287 bp (Figure 1) obtained through multiple sequence alignment was used for designing of *DREB1A* gene-specific primers. The Primer3Plus program predicted five forward and two reverse primers as per the parameters chosen (Table 2a). Out of these, one forward (F1) primer of 19 bp, 52.63% GC content, T_m of 54.21ºC, and absent of secondary structure was chosen. Comparatively, of the two reverse primers, one (R1) primer was found forming a dimer and other (R2) primer was found to have runs of C's and A's in its sequence. Therefore, reverse primer was not finalized. GeneFisher2-primer designing tool resulted both forward and reverse primers. As forward primer was already selected, only reverse primers were taken into consideration. One backward primer R1 of 18 bp, 50% GC content, T_m of 55.18ºC, and absent of secondary structure was chosen (Table 2b). The primer specificity was checked for both F1 and R1 primer by Blast program, and has shown significant alignment with *AtDREB1A* gene.

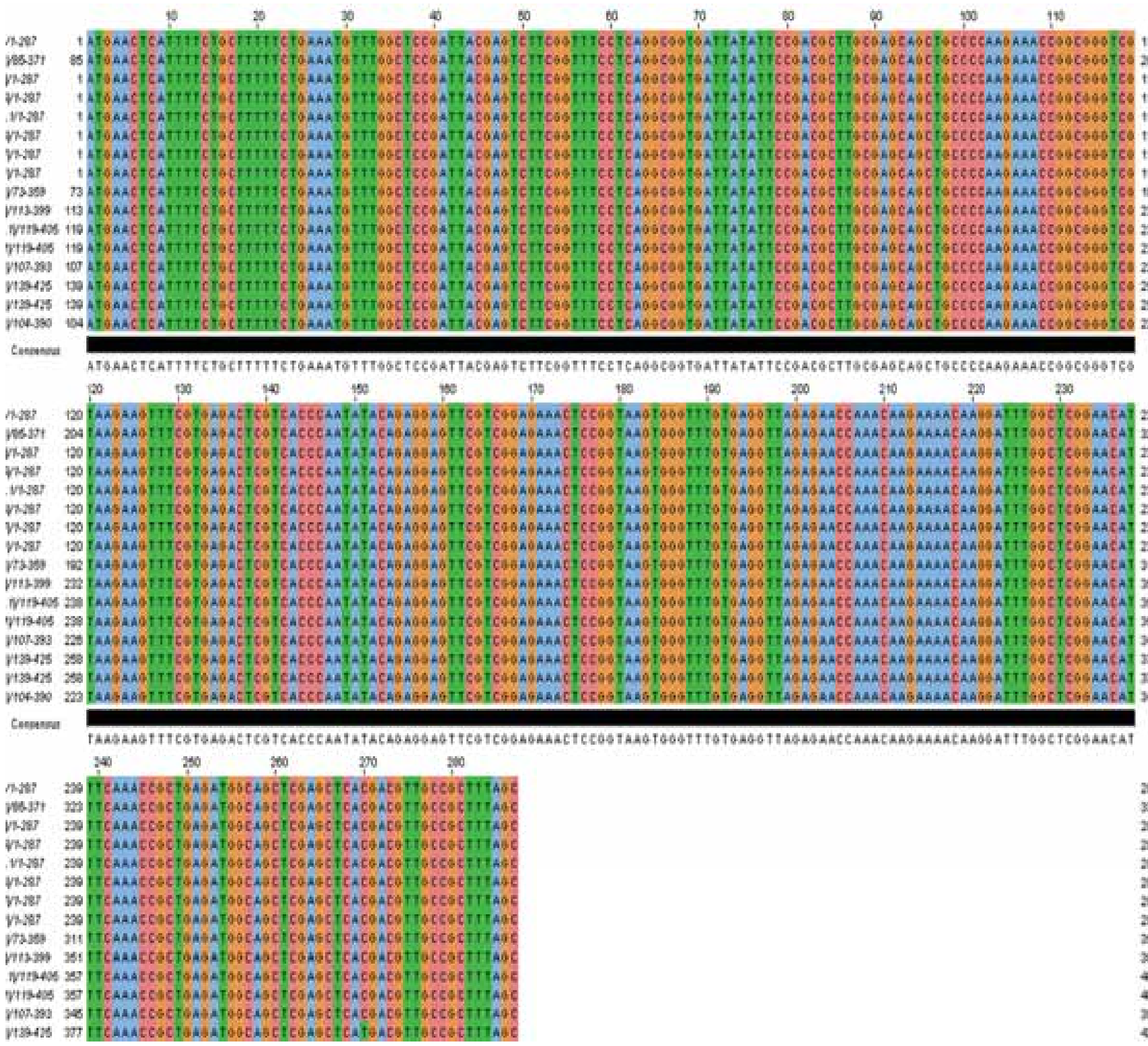

Figure 1. Jalview (www.jalview.org) of Clustalw2 alignment showing consensus sequence of highly conserved (100% identity) domain of 287 bp of *DREB1A* gene sequences from the *A. thaliana* group datamined with NCBI/Entrez

Table 2a. List of primers (Forward & Reverse) predicted by Primer3Plus

Primer Name	Sequence(5'→3')	Length [bp]	Hairpin	Dimer	T_m [°C]	GC%
F1	**GGCTCCGATTACGAGTCTT**	**19**	**0**	**0**	**54.21**	**52.63**
F1	**GGATATATTCCGACGCTG**	**18**	**0**	**0**	**52.2**	**50**
F2	TACGAGTCTTCGGTTTCCTC	20	1	1	54.37	50
F3	TTTGGCTCCGATTACGAG	18	1	1	54.19	50
F4	TGGCTCCGATTACGAGTC	18	1	1	53.56	55.56
F5	GTTTGGCTCCGATTACGA	18	0	0	53.93	50
R1	ACCCACTTACCGGAGTTTCT	20	0	1	55.4	50
R2	ACCTCACAAACCCACTTACC	20	0	0	53.74	50

Table 2b. List of (Reverse) primers predicted by NetPrimer tool

Name of the Primer	Sequence(5'→3')	Length [bp]	Hairpin	Dimer	T_m [°C]	GC%
R1	**ATGTTCCGAGCCAAATCC**	**18**	**0**	**0**	**55.18**	**50**
R2	**GCTGATTGTGATTCCACG**	**18**	**0**	**0**	**52.0**	**50**
R3	GTTCCGAGCCAAATCCTTG	19	1	1	57.85	52.63

3.2 Orthologous Amplification of EcDREB1A Gene Fragment

The template DNA of finger millet cultivars (Section 2.1) produced an amplification of a bright band of ~0.5 kbp (Figure 2). Large-scale amplification of the gene fragment was achieved using the same primer set and was separated in a low melting agarose gel of 1.5%, eluted and purified using Geneaid gel elution kit (www.geneaid.com). The eluted fragment was subjected for DNA sequencing.

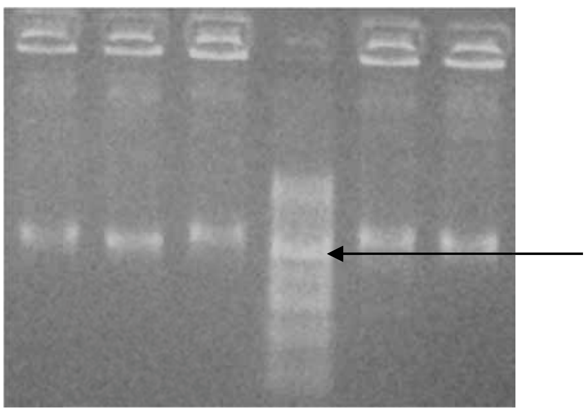

Figure 2. Example of gel electrophoresis of PCR–amplified *EcDREB1A* gene fragment. Shown are ~0.5 Kbp products obtained from 5 different cultivars (Sect.2.1) and DNA ladder marker (M). Position of marker's 500 bp band is indicated by the described arrow

3.3 DNA Sequencing

The amplified *EcDREB1A* gene fragments were sequenced with Applied Biosystems following modified Sanger's Dideoxy method at Chromous Biotech Pvt. Ltd, Bangalore, India and were found to be 536 bp long (Figure 3); it was same case for all the five cultivars of finger millet included in this investigation.

>1.Finger millet (*Eleucine coracana*, cv. Dibyasinha)

CCACATCACTAGGCTGTGATTCTGATCGGACCCCCGCGAGCATCTTCCCCAAGAGACCGG
CGGGGCGTAAGAAGTTACGTGAGACTCGTCGCGAAATTTACAATTGCGCCGCTCGCACAT
ACTACGATAGTGGTGCCTTAGACGTTAGAGACCCAAACACGAAGATGCTCGTTATGCTCG
GTTCCTTTGCAAATCGCTGAGACGACGGACTGGCGCTCAGGACGTTGCCACAAGAGCCTC
TTCGTGGCTGGTCAGCCCGACTCAGTTCAGCAGACTCGTCTTGAAGGCGCCGAATCCTGA
AGTCAACTTCAGATAACAACACTCAATAGGCGTAGGCTGAGTATGGGTAGGAGTGTTGTC
TTGGAATGTCTTAGGCGACGAAGGATCATTTTGATGACATCGAGACCGAAGACTCGTAAG
GGGAGCCACCCGGAGGATCAGAGTAAACTGCGCGTAATAAGCATGATTAGGCGATGGTAG
CGAGTCAGAGACTCCCCGCTTTTATGGCAGCAGGGATGCTTTCGCCGCTTCCGTCC

Figure 3. EcDREB1A gene fragment specific nucleotide sequence derived from amplified and sequenced DNA fragment

3.4 Structural and Phylogenetic Analysis of the Amplified EcDREB1A Gene Fragment

3.4.1 Structural Analysis of Nucleotide Sequence

Table 3. Nucleotide homology of EcDREB1A fragment with orthologous DREB1As datamined by nBLAST analysis of the amplified and sequenced DNA fragment

SlNo.	Accession No.	Sequence Type	Length	Query coverage	e-value(desc.order)	Identity
1	CP002687.1	*Arabidopsis thaliana* chromosome 4, complete sequence	18585056	98%	7e-52	70%
2	FJ169302	*Arabidopsis thaliana* ecotype Nd-1 DRE/CRT-binding factor 3 (CBF3/DREB1a) gene, complete coding DNA sequence	651	98%	7e-52	70%
3	FJ169301	*Arabidopsis thaliana* ecotype Co-1 DRE/CRT-binding factor 3 (CBF3/DREB1a) gene, complete cds	651	98%	7e-52	70%
4	FJ169300	*Arabidopsis thaliana* ecotype Di-G DRE/CRT-binding factor 3 (CBF3/DREB1a) gene, complete cds.	651	98%	7e-52	70%
5	FJ169298	*Arabidopsis thaliana* ecotype Lip-0 DRE/CRT -binding factor 3 (CBF3/DREB1a) gene, complete cds	651	98%	7e-52	70%
6	FJ169297	*Arabidopsis thaliana* ecotype Spr1-2 DRE/CRT-binding factor 3(CBF3/DREB1a) gene, complete cds.	651	98%	7e-52	70%
7	FJ169296	*Arabidopsis thaliana* ecotype Po-0 DRE/CRT-binding factor 3 (CBF3/ DREB1a) gene, complete cds.	651	98%	7e-52	70%
8	FJ169295	*Arabidopsis thaliana* ecotype Gie-0 DRE/CRT -binding factor 3 (CBF3/DREB1a) genes, complete cds.	651	98%	7e-52	70%
9	FJ169294	*Arabidopsis thaliana* ecotype Mt-0 DRE/CRT-binding factor 3 (CBF3/DREB1a) gene, complete cds.	651	98%	7e-52	70%
10	FJ169292	*Arabidopsis thaliana* ecotype Bor-1 DRE/RT-binding factor 3(CBF3 /DREB1a) gene, complete cds.	651	98%	7e-52	70%
11	FJ169291	*Arabidopsis thaliana* ecotype Ta-0 DRE/CRT-binding factor 3 (CBF3/DREB1a) genes, complete cds.	651	98%	7e-52	70%
12	FJ169286	*Arabidopsis thaliana* ecotype Ka-0 DRE/CRT-binding factor 3 (CBF3/DREB1a) genes, complete cds.	651	98%	7e-52	70%
13	FJ169285	*Arabidopsis thaliana* ecotype LP2-2 DRE/CRT-binding factor 3 CBF3/DREB1a) genes, complete cds.	651	98%	7e-52	70%
14	FJ169283	*Arabidopsis thaliana* ecotype Sf-1 DRE/CRT-binding factor 3 (CBF3/DREB1a) genes, complete cds.	651	98%	7e-52	70%
15	FJ169282	*Arabidopsis thaliana* ecotype Ll-0 DRE/CRT-binding factor 3 (CBF3/DREB1a) genes, complete cds.	651	98%	7e-52	70%
16	EF523126	*Arabidopsis thaliana* ecotype Tscha-1 C-repeat binding factor 3(CBF3) mRNA, complete cds	908	98%	7e-52	70%
17	EF523125	*Arabidopsis thaliana* ecotype Sapporo-0 C-repeat binding factor 3 (CBF3) mRNA, complete cds.	908	98%	7e-52	70%
18	EF523124	*Arabidopsis thaliana* ecotype Rsch-0 C-repeat binding factor 3(CBF3) mRNA, complete cds.	908	98%	7e-52	70%
19	EF523122	*Arabidopsis thaliana* ecotype Bur-0C-repeat binding factor 3 (CBF3)mRNA,complete cds	908	98%	7e-52	70%
20	EF523118	*Arabidopsis thaliana* ecotype Litva C-repeat binding factor 3 (CBF3) mRNA, complete cds	908	98%	7e-52	70%
21	EF523115	*Arabidopsis thaliana* ecotype Pog-0 C-repeat binding factor 3 (CBF3)mRNA, complete cds	908	98%	7e-52	70%
22	EF156749	*Capsella bursa-pastoris* DREB1A (DREB1A) gene, complete cds	651	98%	3e-50	70%
23	DQ403814	*Nicotiana tabacum* DREB1A mRNA, complete cds	651	98%	3e-50	70%
24	AY691904	*Arabidopsis thaliana* DREB1A gene, complete cds	805	98%	3e-50	70%
25	XM_002867561	*Arabidopsis lyrata* subsp. lyrata hypothetical protein, mRNA	827	62%	1e-24	70%
26	EF523196	*Arabidopsis lyrata* subsp. petraea C-repeat binding factor 3 (CBF3) mRNA, partial cds	609	69%	1e-24	84%
27	DQ131497	*Iris lactea* var. chinensis CRT/DRE binding factor 1 (CBF1) gene, complete cds.	664	49%	5e-22	72%
28	FJ491243	*Arabis pumila* CBF2 mRNA, complete cds	651	55%	2e-20	70%

Sequence information from the amplified fragment was subjected to homology search using nBLAST algorithm at http://www.ncbi.nlm.nih.gov. The amplified EcDREB1A fragment (536 bp) nucleotide sequence showed 98% query coverage and 70% identity with 651 bp CDSs reported in many of *A. thaliana* sequences (Table 3), with the lowest e-value of 7e-52. This amplified fragment of *EcDREB1A* nucleotide sequence also showed 70% similarity with *Capsella bursa-pastoris* (EF156749), *Nicotiana tabacum* DREB1A sequence (DQ403814) with e-value of 3e-50.The amplified and sequenced nucleotide sequence of partial *EcDREB1A* was submitted to EMBL-ENA which has been accepted with accession No. HF549034 in the said data base.

3.4.2 Structural Analysis of the Predicted *EcDREB1A* Protein Fragment

The obtained amplified genomic sequence was subsequently in silico translated using Expasy translate tool at http://expasy.org/cgi-bin/translate/. The amino acid sequence of *EcDREB1A* gene fragment is shown in Figure 4. The longest detected open reading frame (ORF) of 252 bp is coding for 84 amino acids. The translated *EcDREB1A* fragment encodes a putative protein of 84 amino acids rich in serine (13.10%) with a predicted molecular mass of 9.29 kDa (Figure 4). The amino acid sequence of the obtained *EcDREB1A* fragment showed 88% query coverage and 63% identity with the reported *GhDREB1A* (upland cotton, *Gossypium hirsutum*) DREB sequence, with an e-value of 7e-06. Other cereals, such as *Zea mays* DREBA (53%), *Oryza sativa* DREB1 (55%), *Triticum aestivum* DREB transcription factor 6 (55%), shared varied degrees of identity with the *EcDREB1A* fragment at protein level (Table 5). Multiple sequence alignment of *EcDREB1A* with other amino acid sequences revealed presence of 9 highly conserved amino acids (R, P, G, F, D, R, G, C and N) (Figure 5). However, *EcDREB1A* fragment is phylogenetically separated from other sequences taken for comparison here and forms a distinct orthodox taxonomic unit (OTU) (Figure 6).

```
acggaagcggcgaaagcatccctgctgccataaaagcggggagtctctgactcgctaccat
 R K R R K H P C C H K S G E S L T R Y H
cgcctaatcatgcttattacgcgcagtttactctgatcctccgggtggctccccttacga
 R L I M L I T R S L L - S S G W L P L R
gtcttcggtctcgatgtcatcaaaatgatccttcgtcgcctaagacattcccaagacaac
 V F G L D V I K M I L R R L R H S Q D N
actcctacccatactcagcctacgcctattgagtgttgttatctgaagttgacttcagga
T P T H T Q P T P I E C C Y L K L T S G
ttcggcgccttcaagacgagtctgctggaactgagtcgggctgaccagccacgaagaggc
F G A F K T S L L E L S R A D Q P R R G
tcttgtggcaacgtcctgagcgccagtccgtcgtcctcagcgatttgcaaaggaaccgag
 S C G N V L S A S P S S S A I C K G T E
cataacgagcatcttcgtgtttgggtctctaacgtctaaggcacccactatcgtagtatg
 H N E H L R V W V S N V - G T H Y R S M
tgcgagcggcgcaattgtaaatttcgcgacgagtctcacgtaacttcttacgccccgccg
 C E R R N C K F R D E S H V T S Y A P P
gtctcttggggaagatgctcgcgggggtccgatcagaatcacagcctagtgatgtpbact
 V S W G R C S R G S D Q N H S L V M X T
```

Figure 4. *In silico* translated regions (red) of the amplified finger millet *DREB1A* fragment resultant from Expasy translate tool (http://expasy.org/cgi-bin/translate/)

Table 5. *In silico* translated protein homology of *EcDREB1A* fragment with orthologous *DREB1A*s

Sl No.	Accession No.	Sequence Type	Length	Query coverage	e-value (desc. order)	Identity
1	gb|ABD72616.1	cold resistance related AP2 transcription factor [*Arabidopsis thaliana*]	150	63%	2e-06	37%
2	gb|ABV82985.1|	cold responsive transcription factor [*Thlaspi arvense*]	214	63%	3e-06	35%
3	emb|CAQ52596.1|	DREB1A [*Arabidopsis thaliana*]	216	63%	4e-06	37%
4	gb|ABD65969.1|	DREB1A [*Nicotiana tabacum*]	216	99%	4e-06	37%
5	gb|AAR26658.1|	Cbcbf [*Capsella bursa-pastoris*]	219	63%	5e-06	36%
6	gb|AAP83936.3|	GhDREB1A (upland cotton) [*Gossypium hirsutum*].	216	88%	7e-06	63%
7	gb|AAS00621.1|	DREB1 [*Eutrema salsugineum*]	216	63%	9e-06	35%
8	gb|AEQ35295.1|	DREB1A-like protein [*Lepidium latifolium*].	213	26%	1e-05	59%
9	gb|ACB45087.1|	CRT binding factor 1 [*Solanum habrochaites*]	219	27%	3e-05	58%
10	dbj|BAE17131.1|	LhCBF1 [*Lycopersicon hirsutum*]	222	27%	3e-05	58%
11	gb|ABX00639.1|	DREB1B [*Brassica juncea*]	214	26%	4e-05	56%
12	ref|NP_001170481.1|	DRE-binding protein 4 [*Zea mays*]	232	29%	1e-04	53%
13	dbj|BAL04971.1|	CBF [*Zoysia japonica*]	212	25%	1e-04	55%
14	gb|AAR88363.1|	DREB-like protein 1 [*Capsicum annuum*]	215	24%	1e-04	57%
15	gb|AAY43213.1|	CRT/DRE binding factor 1 [*Hevea brasiliensis*]	231	36%	1e-04	52%
16	ref|XP_002436386.1|	hypothetical protein SORBIDRAFT_10g001620 [*Sorghum bicolor*]	249	39%	1e-04	47%
17	gb|AAM18961.1|	CBF-like protein CBF17 [*Brassica napus*]	250	24%	1e-04	62%
18	gb|AAX28952.1|	HvCBF5 [*Hordeum vulgare subsp. vulgare*]	214	25%	3e-04	55%
19	ref|NP_001056661.1|	Os06g0127100 [*Oryza sativa* Japonica Group]	214	34%	3e-04	55%
20	gb|ABK55356.1|	CBFII-5.2 [*Triticum aestivum*]	219	26%	3e-04	55%
21	gb|ABF59736.1|	CBF-like transcription factor [*Sabal palmetto*]	210	69%	0.002	48%
22	gb|ABF59744.1|	CBF-like transcription factor [*Dypsis lutescens*]	212	64%	0.002	48%

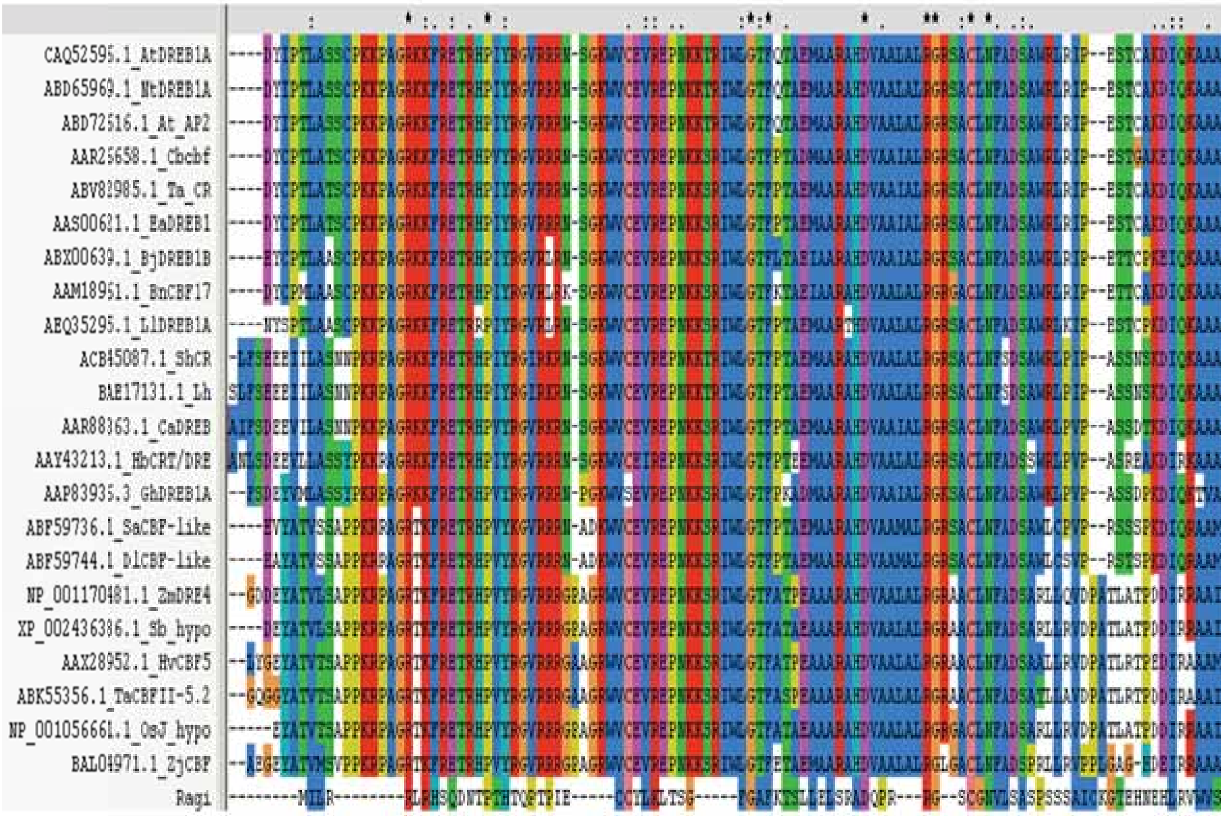

Figure 5. Multiple sequence alignment of 22 orthologous DREB proteins with the obtained EcDREB1A fragment in ClustalX 2.1

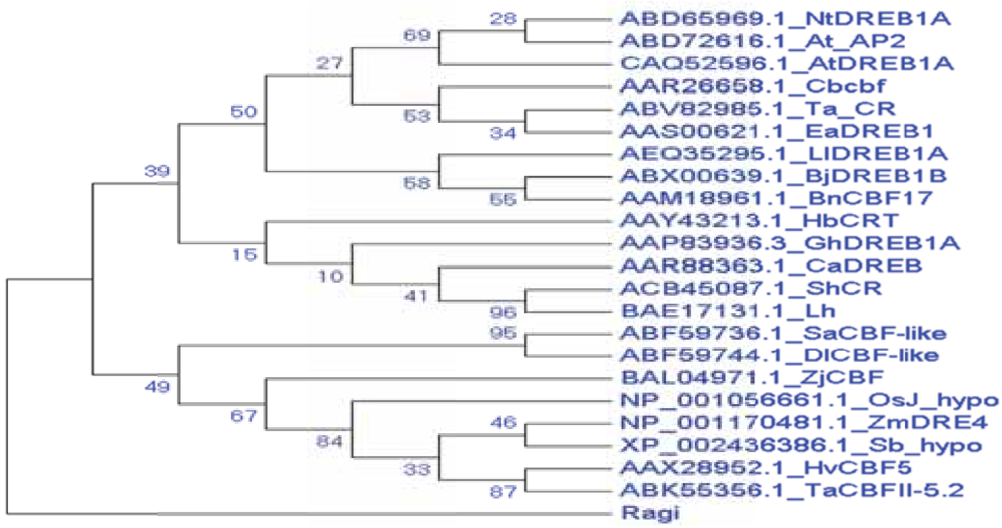

Figure 6. Consensus bootstrap (Maximum Parsimony) tree constructed in MEGA 5.5

4. Discussion

Physiological, biochemical, and genomic tools and techniques have advanced the understanding of plant responses and adaptations to various abiotic stresses like drought, cold, salinity etc. Engineering the stress proteins or the enzymes of the biosynthetic pathways associated with stress responses has been evolving as an encouraging method for improving the abiotic stress tolerance (McCue & Hanson, 1990; Bohnert & Jensen, 1996; Dixon & Arntzen, 1997; Barkla et al., 1999; Blumwald, 2000; Hong et al., 2000).

Dehydration-responsive element (DRE)/C-repeat (CRT) was first identified in *A. thaliana*, as a cis-acting element regulating gene expression in response to dehydration (drought, salinity, & cold stress; Baker et al., 1994; Yamaguchi-Shinozaki & Shinozaki, 1994; Carvallo et al., 2011). Several DRE-binding proteins (DREB)/CRT-binding factors (CBFs) were isolated and identified as key players in dehydration-responsive gene expression (Yamaguchi-Shinozaki & Shinozaki, 1994). *DREB1A* transcription factor seems to be one of the most promising candidate genes involved in conferring the drought tolerance in several crops. Transcriptional regulation is potentially an area for coordinated regulation of genes relevant to stress tolerance, but requires identification of factors limiting the sustained response so that their expression may be manipulated (Xiong & Zhu, 2002). Stress-related genes are found in all plant species. It was hypothesized that genes from hardy plants could have evolved better protection properties and the homologues of *DREB1A* present in different genotypes will be suitable candidate genes for allele mining, integration in the genetic maps to associate them with drought tolerance QTLs, or undertaking candidate gene sequence-based association mapping. Therefore, in the present investigation, afragment of an important transcription factor *EcDREB1A* from finger millet genomic DNA was amplified with species-specific primer pair and characterized based on the sequence information publicly available for different type of *DREB* genes in some model and crop plant species, and using a variety of bioinformatics approaches. Available gene sequences from the model plant *A. thaliana* offer a unique opportunity to clone cognate genes and genes from heterologous sources resulting in discovery of new genes, allele-mining, etc. Primer design and sequence prediction are important steps before synthesizing primers to be used for cognate gene amplification and/or cloning. In the current study, a systematic approach has been made to predict a pair of theoretical primers for the *AtDREB1A* gene for heterologous gene cloning. To achieve this this goal, 16 different but related nucleotide sequences based on *AtDREB1A* gene were retrieved from different public databases. A highly conserved region of 287 bp was detected on multiple alignments through Claustalw2 program and primer set (forward and reverse) was predicted using Primer3plus and NetPrimer software on the basis of the conserved region, considering ideal conditions for primer length, GC content, T_m, formation of primer-dimers, hairpin-loops, etc. The predicted forward and backward primers have shown significant sequence similarity with *AtDREB1A* genes (graphics not shown here) and hence were used for amplification of *EcDREB1A* fragment, possibly leading to discovery of new genes and allele mining.

Finger millet *DREB1* (*EcDREB1A*) genomic sequence fragment was found to be 536 bp long. The similarity search result indicated extensive similarity to other *DREB* genes reported from different plant species suggesting that the amplified fragment belonged to *DREB* class of transcription factors. Significantly high identity of *EcDREB1A* was noticed with *DREB* and its homologs from *A. thaliana, Triticum aestivum*, and *Oryza sativa* (Table 3) Comparative analysis of nucleotide sequences showed 70% similarity and 98% query coverage with the reported *AtDREB2* mRNA, complete CDS, thus indicating the amplified fragment only partially covers the *AtDREB2* sequence. *EcDREB1A* fragment contains a putative ORF of 84 amino acids rich in serine (13.10%) with a predicted molecular mass of 9.29 kDa. Multiple sequence alignment of this *EcDREB1A* ORF with other *DREB* genes revealed presence of 9 highly conserved amino acids (R, P, G, F, D, R, G, C and N).

Allele mining exploits the DNA sequences of one genotype to isolate useful alleles from related genotypes. A significant percentage of allelic polymorphism is known to exist for a given gene and such small differences are implicated in evolutionary relationship between the gene and the trait in question. Such studies could provide the raw materials for plant breeding and translational genomic approaches (Latha et al., 2004; Cavallo et al., 2011). Allele mining of *EcDREB1A* gene across selected five finger millet cultivars revealed no variations on nucleotide, probably due to narrow genetic base among the cultivars taken for current study. Nevertheless, the data generated here indicates the presence of DREB sequence in the plant kingdom. Heterologous gene cloning and allele mining in finger millet with broad genetic base may lead to identification of useful alleles responsible for abiotic stress tolerance.

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Estimation of Phenotypic and Genetic Parameters and Genetic Trend of Weights in the Weaning Phase (P205), Weight at one Year (P365), the Yearling (P505) in Nellore Cattle in the Northern Region and Under-Region Middle-North of Brazil

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Abstract

We studied 257 herds of 257 farms, totaling 79,051 observations in eight States of the Federation, which genetic and phenotypic parameters were estimated for the weights to 205 (P205), 365 (P365) and 550 days old (P550) of Nelore, included in the system of development Control by weight (CDP) conducted by the Brazilian Association of Zebu breeders (ABCZ) and raised in the northern region and sub-region Means North. Estimates of variance components and genetic parameters were obtained by the MTDFREML application, which uses the methodology of the Restricted Maximum Likelihood Derivative Free with an animal model that considers as fixed the effects of the farm such as year, month of birth, and gender. The heredity for P205 was 0.13 ± 0.02 and for P365 was 0.18 ± 0.05, which may indicate little additional genetic variation for these characteristics. For P550 days the estimated heredity was 0.23 ± 0.07, considered average, what indicates that at this age it is possible to select for weight gain. The genetic, phenotypical and environmental likeness among the weights in different ages were: P205-P365 = 0.92, 0.72 and 0.79; P205-P550 = 0.89, 0.61 and 0.87 and P365-550 = 0.98, 0.80 and 0.87. The genetic tendency in P365, for direct effect was significant and positive equals 0.455 kg ($P < 0.001$). Genetic trends in P205, P365 and P550 for direct effect were significant and positive, equal to 0.272 kg ($P < 0.001$), 0.455 kg ($P < 0.001$) and 0.744 kg ($P < 0.001$), respectively.

Keywords: growth, estimated development, genetic changes, zebu cattle

1. Introduction

Beef production in Brazil is characterized by extensive raising, and in this aspect the Zebu breeds and their cross-bred have stood out as the most efficient solution for this kind of system and its use has contributed to the development of livestock, not only meat production, but also production of milk. Although most of Brazilian beef, estimated at more than 220 million animals, concentrate in the Southeast and Midwest, which holds about 62% of the national herd (Ibge, 2003), other regions have a significant quantity of animals.

Among the improvement methods available to modify the genetic potential of the animals, the selection is that one in which, by choosing the parents that will breed the next generation, searches to increase the frequency of desirable genes in the population (Ferraz Filho, Ramos, & Silva, 2002). After the introducing the selection program it is necessary to evaluate it periodically to verify their efficiency (Manson, 1999), as well as evaluate the genetic tendency throughout the time.

In terms of selection, the genetic improvement depends on the knowledge of the heredity of characteristics that interest and likeness among them, for these genetic parameters allow the prediction of direct responses and similar to the selection and the definition of the most appropriate selection method. Thus, the genetic improvement depends on the proper use of genetic variation of the selected characteristics.

In this context, the heredity of a character expresses the rate of the total variance that is attributed to the average effects of the genes, which determine the degree of similarity among the relationship. According to (Falconer, 1987), the most important function of heredity in the genetic study of the metrical character is its power to predict, showing the confidence of the phenotypic value as a guide to the genetic value. In the author's opinion, only the phenotypic value of the individual can be directly measured, but it is the genetic value that determines its influence on the next generation.

Therefore, if the breeder chooses the individuals to cross according to their phenotypic values, the change in the characteristics of the population will be well succeeded and it can be predicted only through the knowledge of the likeness degree between the phenotypic and genetic value that is measured by heredity. (Santos, Gomes, & Silva, 2005) analyzing 11,823 observations in Nellore's breed in Bahia, estimated the heredity for the weight in 365 (P365) and 550 (P550) days old, with respective values for direct and maternal heredity of 0.66 and 0.11 to P365 and 0.57 and 0.07 for P550, suggesting that the selection for both the weights will result in genetic progress, since the coefficients of direct heredity were of very high.

The coefficient of likeness of the environment does not represent exactly the interdependence only because of environmental factors, since the components of variance and (co) variance environment of the similar characteristics include effects of dominance, epistasis and effects of genotype-environment interaction. (Fridrich, Silva, & Figueiredo, 2005), have studied the breed performance, the genetic and environmental factors related to weights at ages of 205 and 365 days in Nellore cattle from the five regions of Brazil and they have found that the estimative of genetic and phenotypical parameters varies a lot from one region to another.

The genetic and environmental likenesses are, frequently, very different in importance, and sometimes different in sign. A signal difference between these two likeness shows that the causes of genetic and environmental variation, affect the characters through different physiological mechanisms (Falconer, 1987).

(Santos, Gomes, & Silva, 2005), analyzing 11,823 observations in Nellore cattle in Bahia, found that the genetic similarity between weight in 365 and weight in 550 days old, was high (0.64), meaning that there are 64% likelihood in similar answer favorable in P550, if the selection is achieved for P365. Currently, the livestock in the North and northeast of Brazil is composed basically by pure Zebu animals or cross-breed created in the pasture, specially for its great adaptation to soil and weather conditions. However, the rates of production in breeding these animals are low and have the lack of wide-ranging genetic improvement programs as one of the reasons (Pied, Malk, & Son Lôbo, 2005).

The aim was to raise the estimates of the components of co-variance to allow the assessment of environmental and genetic parameters for weights of 205, 365 and 550 days of age, and to assess the genetic and phenotypic changes in the weights at 205, 365 and 550 days of age in order to provide data to adopt appropriate criteria to establish breeding programs specific to the northern region and sub-region of the Middle-North, Brazil.

2. Material and Methods

The data analyzed were obtained from the Brazilian Zebu Breeders Association (ABCZ) and refer to Nellore animals included in Weight Development control system (CDP). We studied herds of 257 farms, totaling 79,051 observations. The animals were raised on pasture in the northern region and under-region Middle-North of the country, comprising the States of Amazonas, Pará, Tocantins, Rondônia and Acre and the States of Piauí and Maranhão, respectively. Each three months the animals were weighed until the age of 18 months, beginning the weighing at birth done by the breeder and the others made by ABCZ technicians.

The data obtained by the CDP used to estimate biological and genetic parameters for the weights at 205 (P205), 365 (P365) and 550 days old (P550), was analyzed in the laboratory of Informatics Center of Agrarian Sciences of the Federal University from Piauí (UFPI/CCA). For the organization of contemporary groups (CG) it was considered four periods of birth: January to March, April to June, July to September and October to December. The GC had animals from the same sex, farm, period of birth and year of birth. We have eliminated all the records related to GC with less than five observations. The information on the offspring from mothers over the age of 18 years and less than two years were deleted from the database.

To obtain the estimates of the variances components we used the methodology of Restricted Maximum Likelihood Derivative Free (DFREML), in which we analyzed animal models multi-characterists, using the application

Multiple Trait Derivativ Free Restricted Maximum Likelihood (MTDFREML), developed by (Boldman, Kriese,& Van Vleck, 1995). We used two models: a complete one to estimate genetic parameters and a simple model to estimate additive genetic correlations.

The complete model included random genetic effects, direct and maternal, and permanent environment, in addition to the fixed effect of GC and (co) variable cow age at parturition (years), linear and quadratic effects, admitting the covariance between direct and maternal effects equal to zero (am = 0), considering Y = X $+_1$to Z + Z_2m + Z_3p + e, with (σ_{am} = 0), where Y = characteristics observations vector; X = incidence pattern of fixed effects; = vector of fixed effects; Z_1 = incidence pattern direct additive genetic effect of each animal; a = vector of random direct additive genetic effects; Z_2 = incidence pattern of genetic maternal additive effect of each animal; m = vector of maternal additive genetic random effects; Z_3 = incidence pattern of permanent environment effect of the cow; p = vector of random permanent environmental effects of cow; e = vector of random residual effects; σ_{am} = covariance between direct and maternal random effects. To follow the model it was taken on the following assumptions:

$$E\begin{bmatrix} y \\ a \\ m \\ p \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \qquad VAR = \begin{bmatrix} a \\ m \\ p \\ e \end{bmatrix} = \begin{bmatrix} A\sigma_a^2 & 0 & 0 & 0 \\ 0 & A\sigma_m^2 & 0 & 0 \\ 0 & 0 & I\sigma_p^2 & 0 \\ 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

Where A = pattern of kinship; I = identity pattern; σ_a^2= additive direct genetic variance; σ_m^2 = additive maternal genetic variance; σ_p^2= variance of permanent environment; σ_e^2= residual variance.
For the bivariate analyses, we have taken the following presuppositions:

$$E = \begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 b \\ X_2 b \end{bmatrix} \text{ e}$$

$$VAR = \begin{bmatrix} a_1 \\ e_1 \\ a_2 \\ e_2 \end{bmatrix} = \begin{bmatrix} A\sigma_{a1}^2 & 0 & A\sigma_{a1a2} & 0 \\ 0 & I\sigma_{e1}^2 & 0 & I\sigma_{e1e2} \\ A\sigma_{a1a2} & 0 & I\sigma_{a2}^2 & 0 \\ 0 & I\sigma_{e1e2} & 0 & I\sigma_{e2}^2 \end{bmatrix}$$

Being A = pattern of kinship; I = identity pattern; σ_{a1}^2= additive direct genetic variance forcharacteristic 1; σ_{a2}^2 = additive direct genetic variance for characteristic 2; σ_{a1a2}^2 = additive genetic covariance between direct genetic effects for characteristics 1 and 2; σ_{e1}^2= residual variance to characteristic 1; σ_{e2}^2= residual variance for characteristic 2; σ_{e1e2}^2= covariance between the residual effects for characteristics 1 and 2.

Estimates of genetic, phenotypic and environmental trends for the characteristics were obtained by the average regression of the dependent variable (genetic values, weights observed and the GC's solution) divided by the year of birth by two methods: 1) Linear regression and, 2) Regression by articulated polynomials by using the estimator Spline.

3. Results and Discussion

The estimates of the additive genetic variances ($_{the}\sigma^2$), σresidual ($_r{}^2\sigma$) and phenotypic ($_f{}^2$) and of direct heredity (h 2) and maternal heredity (h $^2{}_m$) for the weights at 205, 365 and 550 days old can be seen in Table 1.

Table 1. Estimates of addictive genetic ($_a{}^2$), residual ($_r{}^2$) and phenotypic ($_f{}^2$) variances and of direct heredity (h^2) and maternal heredity (hm^2), with respective standart-error, for weights at 205 (W205), 365 (W365) and 550 (W550) days old

Weights	σa^2	σp^2	σr^2	σf^2	h^2	h^2_m
(W205)	73,80	54,98	404,62	576,57	0,13±0,02	0,07±0,01
(W365)	213,53	95,74	834,99	1211,67	0,18±0,05	0,06±0,02
(W550)	508	85,05	1506,33	2220,77	0,23±0,07	0,05±0,01

The estimated values for the additive genetic variances were much lower than those related to phenotypic and residual variances indicating that the low genetic variability in the studied herds, is probably due to the prolonged length of stay of breeders and patterns in these herds. Most of the reproductive is coming from the own herd, being used in natural breed. On the other hand, the little use of artificial insemination, limits the possibility of using the animals from other regions of the country.

The additive genetic variance was estimated as lower importance, especially for weight at 205 days and their contribution to the phenotypic variance, at that age, was very low. There has been a rise in the values of the additive variance as the ages of the animals grow. The components of phenotypic variances were different for the three weights, with increase related to the advanced ages (P365 and P550), indicating that, after the weaning, when most of them depend on themselves for food, the animals are more influenced by environmental factors.

The reduced heredity for weight at weaning phase (205 days), indicates little additive genetic contribution, meaning that most of the variation in the characteristics is due to environmental differences among the individuals. The value obtained was less than that reported by Ferraz Filho, Ramos, and Silva (2002) -0.20 ± 0.03-to the Tababuã breed. The estimates of heredity for P365 indicate that there are reasonable additive genetic variation for this characteristic in the herds studied. Thus, the changes in weights is probable at 365 days old by direct selection.

For P550, the value found for heredity estimate (0.23 ± 0.07), must be considered as average indicating that at this age it is possible to make a selection for weight gain, because of the influence of additive genetic factors for this characteristic. A similar result was found by Ferraz Filho, Ramos, and Silva (2002). To Tabapuã breed (0.24 ± 0.03). However, in the literature we can observe a great comprehensiveness for heredity of this characteristic, from 0.09 Lôbo and Martins Filho (2000) up to 0.79 (Rosa, Scott, & Nobre, 1985).

The maternal heredity was considered average and indicates the influence of maternal effect on the development of animals over the time. So, we emphasize the importance of selecting for patterns, cows with good maternal ability and within an ideal age for breeding. Fridrich, Silva, and Figueiredo (2005) estimated the heredity for the direct genetic effect of P205 and P365 in Nellore's breed, in different regions of the country, finding values of 0.01 (South), 0.16 (Southeast), 0.29 (Midwest), (North) 0.21 and 0.16 (Northeast), and of 0.05 (South), and 0.13 (Southeast), 0.29 (Midwest), 0.20 (North) and 0.06 (northeast), respectively. These values, exception of those obtained for the South region, are close to or superior to those found in this study, considering the P205. For P365, the values found by the authors are superior to the ones in this study in the Southeast, Midwest and North regions.

The heredity values were lower in the regions mentioned above, possibly because of the increased environmental variance that was proportionately larger than the direct genetic variance for these regions. The phenotypic, genetic and environmental correlations between the weights to the differentages are shown in Table 2.

Table 2. Correlations estimatites genetic, phenotypic and environmental of for body weights at 205 (W205), 365 (W365) and 550 (W550) days of age

Weight	Rg	Ra	Rf
W205-365	0,92	0,72	0,79
W205-550	0,89	0,61	0,71
W365-550	0,98	0,80	0,87

Estimates of genetic correlations were high and positive between the weights at 205 (P205), 365 (P365) and 550 (P550) days old, indicating that most of the genes that influence the weight at a certain age, also influences the weight in ensuing ages, allowing to infer that the selection to any of the weights should make changes the same way as the others. This is important because it allows the animals to be selected sooner. In General, estimates of correlations are higher for adjacent weights and, as the ages distance, a great amount of these estimates reduce.

The genetic correlation between P205 and P365 was high (0.92), as well as between P205 and P550 (0.89), and even more, between P365 and P550 (0.98), meaning that there is high probability of favorable correlated response, between themselves, probably as a result of pleiotropic effects of genes that act in these characteristics, so that one can perform the selection at 205 days old, expecting that the animals selected will present the same answer at 365 and 550 days old. Similar results were obtained by Seo, Oliveira, and Lôbo (2003), who found genetic correlation equal to 0.93 between P120 and P550 and 0.96 between P455 and P550 for Nellore's breed, in different States in the Southeast and Northeast regions of the country.

The estimate correlations between P205 and P550 were lower than between P365 and P550, recommending that the selection for P550 is performed at one year age. The genetic correlation between P365 and P550, with value of 0.98, means that there are 98% chances of correlated response favorable in P550, if the selection is performed to P365, because there is a high pleiotropic effect of genes that influence in these characteristics. Mucari and Oliveira (2003), studying the Guzerá breed, in the State of MatoGrosso do Sul, found genetic correlations inferior to these mentioned characteristics. Sethi, Oliveira, and Lôbo (2003) found genetic correlations between P120 and P550 equal to 0.93 and between P455 and P550 equal to 0.96, to Nelore breed, in different States of the Southeast and Northeast regions of the country. Although these authors have not worked with P365, the genetic correlation values obtained by them, indicate similar conclusions to those obtained in this study, i.e., positive and high values.

The genetic correlations obtained were similar to those estimated for the same race, in the State of Ceará in the Northeast region by Lôbo and Martins Filho (2000), indicating that the selection to any of the characteristics should provide changes in the others, in the same way. However, these estimates have shown high standard errors, probably due to the reduction in the number of observations at advanced ages, which reflects in most unreliable estimates. The environmental correlations between P205, P365 and P550 indicate that the environmental effects favorable to the weight in a certain age also contribute to a raise in weight at other ages. The values for environmental correlations obtained in this study, probably can be attributed to different and adverse climatic conditions that occur over the months and the years, in the regions studied.

The genetic tendency is a measure that allows evaluating the change brought about by a selection process, in a certain characteristic, over the years (Pied, Malk, & Son Lôbo, 2005). The genetic tendency in P205, for direct effect (Figure 1) was significant and positive equals 0.272 Kg/year. In terms of annual genetic change, it represents 0.16% of the average observed of the population, accumulating reasonable genetic gain in the last 26 years of 7.07 Kg for direct effect. Thus, we noticed that there was a reasonable genetic gain and there was an evolution of the herd over the years.

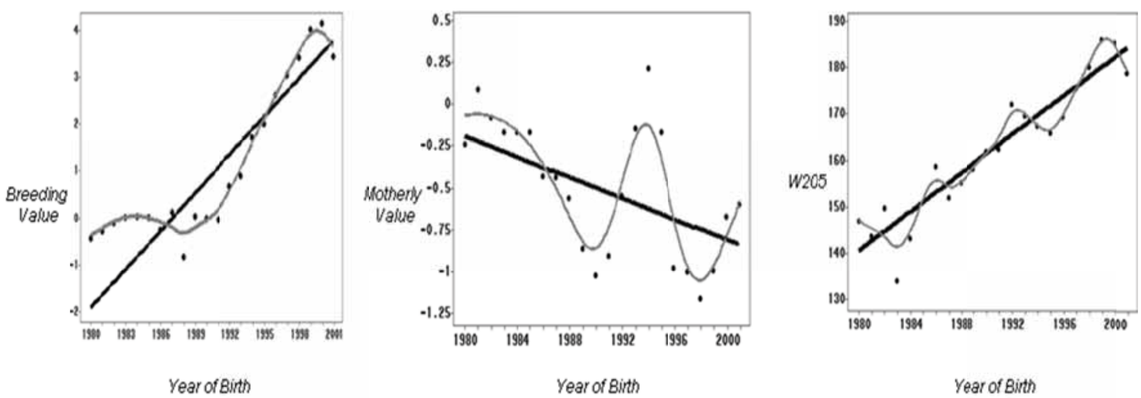

Figure 1. Genetic trends of the direct effect, motherly and fenotipic for 205 days

The genetic trend in P205, to maternal effect was not significant, being equal to 0.031 Kg. In terms of annual genetic change there is no genetic change accumulated by the maternal effect, meaning that there was no proper selection for maternal effect over the years. Similar results were reported by (Azevedo, 2003), studying trends in direct genetic cattle in Tabapuã breed throughout the Northeast. The phenotypic trend presented significant values showing an annual gain of 2.08 Kg/year, reflecting a great evolution over the years.

The genetic tendency in P365, for direct effect (Figure 2) was significant and positive, equal to 0.455 Kg/year. In terms of annual genetic change, this represents 0.20% of the average observed population, accumulating genetic gain in the last 26 years of 11.83 kg for direct effect. The genetic trend in P365, for maternal effect (Figure 2), was significant and equal to 0.252 kg/year. The phenotypic trend resulted in significant values showing an annual gain of 3.155 kg a year, which means a great evolution over the years (Figure 2).

Figure 2. Genetic trends of the direct effect, motherly and fenotipc for365 days

Results indicating low progress are not rare, as those reported by Ferraz Filho, Ramos, and Silva (2002) and (Azevedo, 2003). According to Silva, Rosa, and Gondo (2002), usually, phenotypic changes in the productive characteristics have on environment its greatest contribution. The genetic tendency in P550, for direct effect (Figure 3) was significant and positive equals 0.744 kg/year. In terms of annual genetic change, this represents 0.23% of the average observed population, accumulating genetic gain in the last 26 years of 19.34 Kg for direct effect.

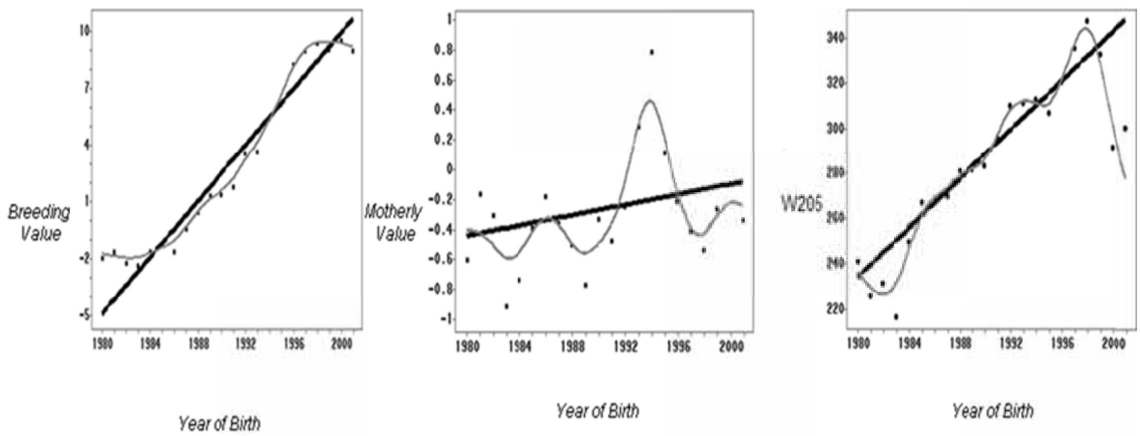

Figure 3. Genetic trends of the direct effect, Motherly and fenotipc for 550 days

The genetic trend in P550, for maternal effect (Figure 3), equal to 0.172 kg a year, was not so significant. Thus, over the years there was no selection for maternal effect. It has to be considered that, at that age, there isn't much influence o the mother on growth. The phenotypic trend presented significant values with an annual gain of 5.440 Kg/year, which represents a great evolution over the years.

According to Biffani, Martins, and Son Giorgetti (1999), in typical livestock conditions in the Northeast, the breeders select the animals much by anatomical and racial characteristics than by productive performance. However, according to Pied, Malk, and Son Lôbo (2005), this trend has been decreasing over the years, as it can be seen in this paper, in which, it has occurred a small genetic gain over the years of study corroborating with these authors and also with Santos Gomes, and Silva (2005).

4. Conclusions

Animal selection for weight is more efficient at more advanced ages, because in these selections heredity estimates are higher. It is possible to anticipate the selection of animals to the weaning without damage to the yearling weight. There was little genetic gain over the analyzed years which points to the selection more focused on the productive performance.

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Improved Method for Estimating Soil Moisture Deficit in Oil Palm (*Elaeis guineensis* Jacq.) Areas With Limited Climatic Data

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Abstract

Widespread water deficit is expected to depress growth and yields of oil palms, which sustain daily losses of water through evapotranspiration of 4-5 mm. While accurate predictions for soil moisture deficit are essential for supplying water to plants through irrigation when rainfall is insufficient, soil moisture deficit is difficult to assess. In Malaysia (as in other oil palm-growing countries) rainfall and rain days are the sole climatic parameters recorded; this limited information is insufficient for reliable estimates of water deficit. This paper reports the adoption of a new method of prediction for soil moisture deficit that takes into account the effective rainfall, evapotranspiration, and a correction factor for the water-holding capacity of the soil. Monthly effective rainfall varied from 11% of gross rainfall to 84%. The number of months with soil water deficit varied from 2 to 12 (mean = 9). Values of water deficit obtained were close to those calculated using the Penman equation, thus validating this method of prediction of water deficit.

Keywords: soil water deficit, effective rainfall, evapotranspiration, available water holding capacity, oil palm area, irrigation

1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) grows naturally and is cultivated in the humid tropics of Africa, Latin America and Asia. Its growth and yield are highly correlated to the availability of water, to compensate for water loss through evapotranspiration. The main natural source of water is rainfall, which is limited from time to time. Therefore, it is necessary to determine the water deficit of the soil in the oil palm-growing area so any shortfall can be provided through irrigation.

Water contributes to the integrity of plant cells where it is a solvent for mineral nutrients and foodstuffs to be translocated throughout the plant (Slatyer, 1967). Plants extract water contained in the soil moisture reservoir, which has been defined by Wadleigh et al. (1965) as the inches of available water that may be held per foot of depth multiplied by the depth of rooting of the plant. This water is provided to the soil moisture reservoir by rains. Topping up of the soil moisture reservoir over the course of the cropping season depends on the amount and frequency of precipitation, the nature of the soil and the condition at the soil surface, all of which affect the rate of infiltration, as well as on the management of the soil reservoir for efficient use of water and nutrients. Often, precipitation is unevenly distributed both in terms of timing and location (Renne, 1965), leading to soil water deficits. According to Slatyer (1967), water deficiency (i) reduced the rates of cell division and cell elongation, which affect plant growth; (ii) accelerated breakdown of RNA; (iii) effected changes in total nitrogen, protein nitrogen and amino acids levels; and (iv) led to limited photosynthesis resulting from poor supply of CO_2, reduced utilisation of light energy and low fixation of CO_2.

The optimum rainfall for oil palm is 2000 mm per year. However, the plant can grow and yield well in areas receiving rainfall of at least 1800 mm, provided it is evenly distributed throughout the year without any chronic water deficit in the soil (Hartley, 1988), congruent with Surre (1968) who estimated that a total of 1800-2000 mm of annual precipitation would ensure good foliar emission, a satisfactory production level of number of bunches and a substantial average bunch weight. Water stress is associated with high incidence of juvenile, fused pinnae and retarded growth of seedlings that are discarded at the end-of-nursery culling (Sime & Darby, 2011). Water stress reduces photosynthesis and practically stops oil palm growth (Corley, 1976; Ochs & Daniel, 1976). An annual water deficit of around 700 mm presents a major risk of mortality for oil palms during dry periods in Benin (Chaillard et al., 1983). Symptoms of water stress are gradual in oil palm grown in West Africa. The first stage is the accumulation of 5 to 6 unopened spears rather than the 2 to 3 under optimum moisture circumstances and 2 or 3 green snapped leaves. In the second stage, in addition to the snapping of 4 to 6 leaves, bunches dry out with failure of some of the fruits to achieve ripeness. The drying out of all the leaves at the base of the crown and the toppling of the upper part of the canopy which is comprised of the meristem characterise the third stage. The final stage is the death of the tree (Maillard et al., 1974). Accumulation of unopened spears and yellowing and snapping of leaves are the most evident symptoms of water stress for oil palm in Costa Rica (Villalobos et al., 1992). However, moisture stress and excessive rain during the wet season can also contribute to depressing oil palm yield through the reduction of evapotranspiration and photosynthesis (Kee et al., 2000). The extent of the water deficit depends on the water retention capacity of the soil. In Benin where oil palm was the main cash crop, precipitation was less than 1200 mm with 4-5 months of drought (Hartley, 1977), but water stress was moderate and did not exclude oil palm cultivation thanks to the high water retention capacity of the soils (Purseglove, 1972).

In oil palm, the sex-differentiation is strictly water-dependent. Moisture stress induces the abortion of female inflorescences, thereby depressing bunch yield. In West Africa, studies on the impact of water stress on oil palm yield showed that an increase of 100 mm reduces the oil-to-mesocarp ratio (Ochs & Daniel, 1976). Several measures of improving soil moisture during prolonged dry season have been tried, but unfortunately they were found to lead to less than spectacular yield increase in comparison with irrigation (IRHO, 1969). In fact, only irrigation has assured returns to farmers by permitting the establishment of highly productive agriculture in areas where rainfall is inadequate or unreliable. In oil palm, fresh fruit yields of 26 t/ha have been obtained in the Ivory Coast with irrigation compared to 7.5 t without irrigation (Desmarest, 1967). Irrigation work carried out by IRHO (Institut de Recherches pour les Huiles et Oléagineux) showed that a supply of 1 mm of water increases yield by 26 kg/ha /tree, i.e. 13.5 t/ha for a minimum daily supply of 4 mm (IRHO, 1969). Corley's (1996) review of various irrigation projects in oil palm cultivation found an increase in fruit bunch yield of 50% , on average, over the control, mostly due to increased bunch number (33%), irrespective of the irrigation system. But the increment of mean bunch weight was relatively low (8%). In Malaysia, Roslan, and Haniff (2004) obtained an increase in fruit bunch yield of 14% at the water rate of 120 l/oil palm against 23% at 240 l/oil palm.

One determining factor governing the success of the irrigation in oil palm is a good prediction of the prevailing water deficit of the soil, i.e. the quantity of water to be supplied via irrigation. Using the Penman equation of calculation of ETc (Evapotranspiration) provided reliable estimates of water deficit in the assessment of current and potential yield from oil palm (*Elaeis guineensis* Jacquin) cultivation in the coastal zone and in N'gusti in Cameroon (Bakoumé, 1987, 2011). Similarly satisfactory results were obtained in the assessment of changes in evapotranspiration from an oil palm stand exposed to seasonal soil water deficits in La Me (Côte d'Ivoire) using the Penman equation (Dufrene et al., 1992). The Penman equation-based estimates of soil moisture deficit were comparable to those derived from a lysimeter in Malaysia (Corley, 1996). The Penman equation requires detailed meteorological data including (i) temperature (minimum, maximum, average), (ii) rainfall, (iii) rain days, (iv) relative humidity, (v) wind speed, (vi) ratio of day wind speed-to-night wind speed, (vii) saturation vapour pressure, (viii) extra-terrestrial radiation, and (ix) mean daily maximum possible sunshine hours, to name a few. Currently, in Malaysia, as in most oil palm areas, only rainfall and number of rain days are recorded. The Penman equation which had provided good estimates of the water deficit was abandoned mainly due to limited available climatic data. Therefore, simpler methods based on the rainfall and rain days only are commonly used in which evapotranspiration in oil palm area is assumed (IRHO, 1969). Effective rainfall (ER) is derived from the gross rainfall minus [runoff + deep percolation + interception by the vegetation] (Kee et al., 2000). Interception is subjective because water interception depends on the age of the oil palm and the management of the foliage (pruned or non-pruned). Furthermore, ER does not take into account the fact that only a fraction of the available water-holding capacity is effectively accessible to the oil palm. Other studies simply use the gross rainfall in the calculation of the soil water deficit (Univanich irrigation project, cited by Corley, 1996).

Any reliable method for assessment of soil water deficit would have to generate values comparable to those generated by Penman equation-based calculation. Effective rainfall, one of the two factors used in the calculation of the soil water deficit, is well predicted by a formula which allows the integration of more factors contributing to the rainfall losses. In addition, the method would best incorporate the available water holding capacity (AWHC) of the soils in the assessment of ER. Evapotranspiration (ETc) calculated using the Penman equation, must be corrected using the cropping factor for application to the oil palm area. The method used in the current work envisages reducing the gaps between values of water deficit obtained and those that could have been generated using the Penman equation in the calculation of the evapotranspiration. Therefore, it takes into account the effective rainfall, the available water holding capacity of the oil palm area, and the cropping factor.

2. Materials and Methods

Climatic data and soil available water holding capacities (AWHC) belonging to twelve oil palm areas from Malaysia and one from Cameroon were used to calculate water deficits. The 13 oil palm areas were regularly subjected to two to three consecutive dry months per year. In the current work, a dry month is a month which receives less than 125 mm/month of rainfall with more than 60% probability of occurrence. In Malaysia, one or two oil palm areas were selected from each of the following states: Johor, Kedah, Melaka, Pahang, Perak, Selangor, and Negeri Sembilan. Rainfall and rain days were averaged monthly over 12- to 24 year-records with the exception of Johor's oil palm area, for which records cover 8 years. Values of AWHC were those obtained by Foster (1984) and Mathew et al. (2006) for different soil series and soil groups in Malaysia.

The water deficit was obtained by comparing the potential evapotranspiration of oil palm (ETc) to efficient rainfall (ER). IRHO's predicted ETc was used because rainfall and rain days were the only climatic parameters recorded and available. In this respect, ETc was considered to be 150 mm/month in a month with less than 10 days of rain and 120 mm/month for 10 days and above of rain, as in previous work by Chaillard et al. (1983), Corley (1996) and Roslan and Haniff (2004). The cropping factor allows conversion of evapotranspiration obtained using the Penman equation; applicable for soils covered with a uniform cover of grass to evapotranspiration in an oil palm area was considered to be one (1.00) (Sys et al., 1978). Monthly ER values were calculated as a function of the rainfall (Rm) and ETc in a given month, according to the equation developed by Troch (1986). A correction factor (*f*) was applied to take into account the AWHC of the soils of the oil palm area. The correction concept is based on the fact that only 2/3 of the water from the soil water reserve is accessible to the oil palm (Sys et al., 1978). The value of *f* is equal to one (1) when AWHC is 75 mm. *f* varies from 0.73 when AWHC is 20 mm to 1.08 when AWHC is 200 mm. Therefore, Troch's equation for calculation of ER was formulated after improvement as follows:

$$ER_{(AWHC)} = [Rm^2 \text{ x } (0.025/ETc - 0.001) + Rm \text{ x } (0.6 + 0.0016\ ETc)] \text{ x } f$$

where ETc is evapotranspiration of oil palm in the month; Rm is the rainfall value of the month; and *f* is the correction factor dependent on AWHC.

The rainfall losses (L_r) through surface runoff, interception by vegetation (precisely the oil palm tree canopy) and infiltration into the soil, to name a few, were calculated as a function of Rm as follows:

$$L_r = [(Rm-ER)/Rm] \text{ x } 100$$

where Rm is the rainfall value of the month, and ER is the effective rain in a given month.

N'gutsi, a West Cameroon oil palm area, was incorporated in the current study. In a previous study of water deficit in this area, more detailed climatic parameters recorded over 23 years in addition to rainfall and rain days were available. With such an advantage, the Penman equation (FAO, 1976) was used to predict ETc. N'gusti was included in the work to enable comparison of water deficit values generated by Penman equation-based evapotranspiration to those generated by the new improved method for use in the case of limited available climatic data.

3. Results and Discussion

3.1 Effective Rainfall

Effective rainfall refers to the percentage of rainfall which becomes available to plants and crops, i.e. oil palms in the case of the current study. ER and L_r values for the different oil palm areas are shown in Table 1. Water losses (L_r) ranged from 16% in Bagan Datoh (Perak) in June and August with 72 mm and 76 mm of rainfall, respectively, with a soil's AWHC estimated at 213 mm to 89% in N'gusti (West Cameroon) in August, the rainiest month (865 mm) with only 90 mm of AWHC. Globally, in absolute terms, rainfall losses were low in areas with relatively high AWHC for comparable monthly (or annual) rainfalls. N'gusti (West Cameroon) and Bukit Badong (Selangor) received the same amount of rainfall (208 mm) in June and in April, but values of L_r were 37% and 32%, respectively. The difference was probably related to differences in the AWHC of their respective soils (90 mm in N'gusti vs. 253 mm in Bukit Badong). October in Bukit Badong (Selangor) and May in Main (Kedah) is another example of oil palm areas with comparable rainfall but different values of L_r. Similarly, Ayer Tekah (Melaka) with an annual rainfall of 1319 mm and an AWHC of 77 mm recorded relatively higher rainfall losses than did Bagan Datoh (Perak) with an annual rainfall 1477 mm and an AWHC of 213 mm.

Table 1. Effective rainfall and rainfall losses in different oil palm areas

State	Area name	AWHC (mm)	Climatic parameter	Month												Year total
				Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Johor	Simpang Kiri	100	Rainfall (mm) (8 yr)	160	49	159	117	112	120	145	88	100	131	149	177	1506
			Rain days (8 yr)	10	7	12	12	11	11	13	10	11	14	15	12	136
			ER (mm)	108	40	108	83	80	86	100	65	72	92	102	117	1055
			L_r (%)	32	18	32	29	28	29	31	26	27	30	31	33	30*
Kedah	Main	98	Rainfall (mm) (24 yr)	85	120	190	267	266	194	199	235	320	385	292	166	2720
			Rain days (24 yr)	7	7	13	15	14	12	12	15	17	20	16	11	159
			ER (mm)	67	90	124	158	158	127	129	145	176	191	167	112	1644
			L_r (%)	22	25	35	41	41	35	35	38	45	50	43	33	40*
	Katumba	98	Rainfall (mm) (11 yr)	77	78	182	211	212	197	215	241	269	311	243	130	2367
			Rain days (11 yr)	5	5	9	11	11	10	11	12	13	17	15	9	128
			ER (mm)	61	62	129	136	136	129	138	149	161	175	150	98	1524
			L_r (%)	20	20	29	36	36	34	36	38	40	44	38	25	36*
Melaka	Ayer Tekah	77	Rainfall (mm) (24 yr)	93	59	133	127	99	82	76	73	74	138	197	167	1319
			Rain days (24 yr)	6	4	9	9	7	6	6	7	7	9	11	9	92
			ER (mm)	71	47	97	93	75	63	59	57	58	100	125	117	962
			L_r (%)	24	21	27	27	24	23	22	22	22	28	36	30	27*
Pahang	Mentakab	97	Rainfall (mm) (24 yr)	135	103	155	180	167	129	117	138	180	195	250	226	1974
			Rain days (24 yr)	9	6	9	9	9	7	8	8	10	11	13	13	111
			ER (mm)	100	79	112	126	120	96	88	102	119	127	152	141	1363
			L_r (%)	26	23	27	30	29	25	24	26	34	35	39	37	31*
	Lanchang	97	Rainfall (mm) (19 yr)	108	97	173	151	155	118	141	129	158	210	254	178	1873
			Rain days (19 yr)	9	6	9	8	8	7	8	8	9	11	13	12	110
			ER (mm)	83	75	123	110	112	89	104	97	114	134	153	118	1312
			L_r (%)	23	23	29	27	27	24	26	25	28	36	40	34	30*
Perak	Bagan Datoh	213	Rainfall (mm) (24 yr)	118	97	148	126	94	72	84	76	153	160	196	154	1477
			Rain days (24 yr)	8	6	9	8	6	5	6	7	9	11	12	10	99
			ER (mm)	94	79	115	100	77	60	70	64	118	115	135	111	1138
			L_r (%)	20	18	23	21	18	16	17	16	23	28	31	28	20
	Melentang	213	Rainfall (mm) (24 yr)	139	107	130	143	108	85	97	108	134	169	198	184	1602
			Rain days (24 yr)	8	7	8	8	7	6	6	7	9	10	12	11	100
			ER (mm)	109	87	103	111	87	70	80	87	105	120	136	128	1225
			L_r (%)	22	19	21	22	19	17	18	19	21	29	31	30	24*
Selangor	Bukit Badong	253	Rainfall (mm) (24 yr)	183	140	194	208	183	96	128	162	184	264	305	220	2268
			Rain days (24 yr)	8	6	9	10	7	5	6	7	9	10	13	9	98
			ER (mm)	136	110	142	141	136	79	101	123	136	166	181	156	1608
			L_r (%)	26	22	27	32	26	18	21	24	26	37	41	29	29*
	Bukit Belimbing	253	Rainfall (mm) (24 yr)	185	125	150	133	87	94	112	157	178	219	262	243	1949
			Rain days (24 yr)	10	6	8	8	6	6	6	7	8	11	12	11	99
			ER (mm)	129	100	116	105	72	77	90	120	133	146	166	157	1413
			L_r (%)	30	21	23	21	17	18	19	23	25	33	37	35	27*
Negeri Sembilan	Main	81	Rainfall (mm) (12 yr)	144	64	205	159	176	122	120	106	150	194	258	214	1912
			Rain days (12 yr)	9	5	10	9	9	7	7	7	9	11	14	11	107
			ER (mm)	103	50	129	112	122	90	89	80	107	124	152	133	1292
			L_r (%)	28	21	37	29	31	26	26	25	28	36	41	38	32*
	Kelamah	81	Rainfall (mm) (13 yr)	120	58	153	134	102	103	97	109	104	157	204	199	1540
			Rain days (13 yr)	8	3	9	8	6	7	8	8	8	10	12	10	96
			ER (mm)	89	46	109	98	77	78	74	82	78	111	137	134	1112
			L_r (%)	26	21	29	27	24	25	24	25	25	29	33	33	28*
West Cameroon	N'gutsi	90	Rainfall (mm) (23 yr)	59	80	208	319	460	552	840	865	703	580	318	99	5084
			Rainy days (23 years)	4	6	14	17	19	22	24	27	25	23	17	6	205
			ERc (mm)	47	62	132	174	199	198	108	93	167	195	174	76	1625
				52	*70*	*162*	*217*	*251*	*221*	*125*	*125*	*190*	*230*	*208*	*83*	*1934*
			L_r (%)	20	22	37	46	57	64	87	89	76	66	45	24	68*

AWHC: available water holding capacity; ER: Effective rainfall; L_r (%): percentage of rainfall loss; *: mean annual rainfall loss; Note: Daily potential evapotranspiration is 5 mm if they are less than 10 rain days in the month and 4 mm if 10 rain days or more; In italics are N'gusti's values of ER derived from Penman equation.

Mean annual L_r varied from 20% in Bagan Datoh (Perak) to 68% in N'gusti (West Cameroon). It ranged from 20% to 40% (16% to 50% monthly) when only Malaysian oil palm areas were considered. The range of rainfall losses found in this work was wider than that (35-40%) obtained by Kee et al. (2000). If the high rainfall – which could be an additional explanation for high rainfall losses – in N'gusti (West Cameroon) were to be excluded from the analysis, the ultimate reason for the wide range of water losses was the wide range of AWHC across all oil palm areas, which this current method of estimation of ER took into account.

Results obtained showed that ER depends on rainfall intensity. Monthly rainfall beyond 200 mm resulted in water losses between 35% and 89%; that is to say, they resulted in less effective rainfall. In fact, such excessive rainfall is harmful as it hinders plant growth and subsequently crop yields by leaching nutrients (Hillel, 1971). Other features such as the duration of the rainfall, the terrain, the initial water content of the soils, all of which also affect the effective rainfall, were not taken into consideration in this work as well as almost all previous works. High initial water content of the soil could have led to lessening of the effective rainfall. The difference between values of ER in N'gusti obtained in previous work (indicated by italics in Table 1) and those obtained in the current study may be due to the fact that, in the previous work, ETc was calculated taking into account climatic factors such as radiation, temperature, humidity, and day and night wind speeds, to name a few, which control plant transpiration.

3.2 Water Deficit

Water deficit is a result of an interaction between soil water (mostly provided by rainfall), atmospheric demand (evaporation) and the plant (transpiration) (Shaw & Laing, 1965). Water deficits in different oil palm areas studied are presented in Table 2. In the current work, a water deficit was found in a month when the ETc was greater than the ER. The method of prediction of water deficit reported in this paper showed that oil palm could have received insufficient water for a considerable number of months in a year. The number of months with water deficit varies from 2 in Main (Kedah) to 12 in Simpang Kiri (Johor) (mean = 9). Concurrently, in Malaysia, in absolute terms, the lowest annual water deficit (143 mm) was recorded in Main (Kedah) and the highest (808 mm) in Ayer Tekah (Melaka) over 11 months. Water deficit revealed in N'gusti (West Cameroon) (136 mm) over 4 months was close to the 152 mm obtained using the Penman equation in the calculation of ETc. In fact, water deficit is in line with the ER calculated based on the prediction of an ETc of 150 mm/month if there were fewer than 10 rain days in the month and 120 mm if there were 10 or more rain days. Surre (1968) recognized that IRHO's method of prediction of water deficits when data on only rainfall and rain days are available was useful in the determination of the water balance. Furthermore, the method used in this work is more holistic than the IRHO's method because it takes into account the AWHC, which is correlated both with soil texture (Foster 1984) and with soil structure (Bakoumé, 1987). Surre and Ziller (1963) have found that the available water domain remains constant where the content of fine soil particles varies from 20 to 50% and also when the suitable texture corresponds in general to 25-30% content of fine particles. Differences in clay and silt rates between horizons or between profiles translate into variations in water content between horizons on the one hand and between profiles on the other.

Results revealed that although Simpang Kiri (Johor) and Kelamah (Negeri Sembilan) recorded comparable mean annual precipitations of 1506 mm and 1540 mm, respectively, the water deficit in Kelamah (574 mm) represented 140% of that of Simpang Kiri. This phenomenon could result from the 10 months with 10 or more rain days experienced in Simpang Kiri versus only 3 months with 10 or more rain days in Kelamah. On the same note, Bukit Badong (Selangor) and Main (Negeri Sembilan) with comparable rainfall (1949 mm vs. 1912 mm) and 4 months with 10 or more rain days each but very distinct AWHC (253 mm vs. 81 mm) recorded almost the same water deficits (377 mm vs. 380 mm). Thus, water deficit was shown to be dependent on number the rain days per month. For the same annual rainfall, water deficit was high in oil palm areas where the number of rain days recorded was low, an indication of the extent to which the rain is evenly distributed throughout the year. The higher the number of rain days, the better the rainfall distribution. The method of water deficit prediction used in the current work supports the finding of Bakoumé (1987) from a similar study using a modified Penman equation for determination of ETc in the estimation of water deficit. That study found that water deficit was indicative of a good or a bad distribution of precipitation throughout the year. Good rainfall distribution leads to low water deficit.

Table 2. Water deficits in different oil palm areas studied

State	Area name	AWHC (mm)	Climatic parameter	Month												Annual water deficit (mm)
				Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Johor	Simpang kiri	100	ETc (mm)	150	150	120	120	120	120	120	120	120	120	120	120	
			ER (mm)	108	40	108	83	80	86	100	65	72	92	102	117	
			Water balance	-12	-110	-12	-37	-40	-34	-20	-55	-48	-28	-18	-3	413
Kedah	Main	98	ETc (mm)	150	150	120	120	120	120	120	120	120	120	120	120	
			ER (mm)	67	90	124	158	158	127	129	145	176	191	167	112	
			Water balance	-83	-60	4	38	38	7	9	25	56	71	47	-8	143
	Katumba	98	ETc (mm)	150	150	120	120	120	150	120	120	120	120	120	150	
			ER (mm)	61	62	129	136	136	129	138	149	161	175	150	98	
			Water balance	-89	-88	9	16	16	-21	18	29	41	55	30	-52	176
Melaka	Ayer Tekah	77	ETc (mm)	150	150	150	150	150	150	150	150	150	150	120	150	
			ER (mm)	71	47	97	93	75	63	59	57	58	100	125	117	
			Water balance	-79	-103	-53	-57	-75	-87	-91	-93	-92	-50	5	-33	808
Pahang	Mentakab	97	ETc (mm)	150	150	150	150	150	150	150	150	150	120	120	120	
			ER (mm)	100	79	112	126	120	96	88	102	119	127	152	141	
			Water balance	-50	-71	-38	-24	-30	-54	-32	-18	-1	7	32	21	326
	Lanchang	97	ETc (mm)	150	150	150	150	150	150	150	150	150	120	120	120	
			ER (mm)	83	75	123	110	112	89	104	97	114	134	153	118	
			Water balance	-67	-75	-27	-40	-38	-61	-46	-53	-36	14	33	-2	398
Perak	Bagan Datoh	213	ETc (mm)	150	150	150	150	150	150	150	150	150	120	120	120	
			ER (mm)	94	79	115	100	77	60	70	64	118	115	135	111	
			Water balance	-56	-71	-35	-50	-73	-90	-80	-86	-32	-5	15	-9	573
	Melentang	213	ETc (mm)	150	150	150	150	150	150	150	150	150	120	120	120	
			ER (mm)	109	87	103	111	87	70	80	87	105	120	136	128	
			Water balance	-41	-63	-47	-39	-63	-80	-70	-63	-45	0	16	8	485
Selangor	Bukit Badong	253	ETc (mm)	150	150	150	120	150	150	150	150	150	120	120	150	
			ER (mm)	136	110	142	141	136	79	101	123	136	166	181	156	
			Water balance	-14	-40	-8	21	-14	-71	-49	-27	-14	46	61	6	201
	Bukit Belimbing	253	ETc (mm)	120	150	150	150	150	150	150	150	150	120	120	120	
			ER (mm)	129	100	116	105	72	77	90	120	133	146	166	157	
			Water balance	9	-50	-34	-45	-78	-73	-60	-30	-17	26	46	37	377
Negeri Sembilan	Main	81	ETc (mm)	150	150	120	150	150	150	150	150	150	120	120	120	
			ER (mm)	103	50	129	112	122	90	89	80	107	124	152	133	
			Water balance	-47	-100	9	-38	-28	-60	-61	-70	-43	4	32	13	380
	Kelamah	81	ETc (mm)	150	150	150	150	150	150	150	150	150	120	120	120	
			ER (mm)	89	46	109	98	77	78	74	82	78	111	137	134	
			Water balance	-61	-104	-41	-52	-73	-72	-76	-68	-72	-15	9	6	574
West Cameroon	N'gusti	90	ETc (mm)	150	150	120	120	120	120	120	120	120	120	120	150	
			ERc (mm)	47	62	132	174	199	198	108	93	167	195	174	76	
			Water balance	-103	-88	12	54	79	78	-12	-27	47	75	54	-74	136
				-136	*-124*	*-45*	*7*	*45*	*66*	*2*	*21*	*34*	*56*	*18*	*-97*	*152*

AWHC: available water holding capacity; ETc: potential evapotranspiration of the crop (oil palm); ER: Effective rainfall; Note: Daily potential evapotranspiration is 5 mm if they are less than 10 rain days in the month and 4 mm if 10 rain days or more; In italics are N'gusti's values of annual water deficit derived from a modified Penman equation for calculation of ETc.

Oil palm areas in Simpang Kiri (Johor) and Main (Kedah) recorded similar rainfall distribution and their soils had a similar AWHC (100 mm vs. 98 mm), but the water deficit was higher in Simpang Kiri compared to that in Main (413 mm vs. 143), in absolute terms. The most relevant feature was found to be the rainfall, which was relatively higher in Main (2720 mm vs. 1506 mm). The method of estimation of water deficit used in the work demonstrated that water deficit depends not only on the rainfall distribution but also on the rainfall intensity. High rainfall intensity results in low water deficit. Katumba (Kedah) and Bukit Badong (Selangor) received almost the same rainfall in October and November (311 mm vs. 305 mm, respectively), but the water reserve (positive value of ER-ETc) was higher in Bukit Badong despite the good rainfall distribution in Katumba (17 rain days in Katumba vs. 13 in Bukit Badong) because of a high AWHC of the soil in Bukit Badong (253 mm vs. 97 mm). In Lanchang (Pahang) and Main (Negeri Sembilan) with same rainfall intensity in October (254 mm vs. 258 mm) and with a slightly better distribution in Main (14 rain days vs. 13), there was a slightly higher water reserve in Lanchang (14 mm vs. 4 mm) due to the relatively higher AWHC of its soils (97 mm vs. 81 mm). It was observed that although water deficit depended on AWHC of the soil of the oil palm area, water deficit was in fact a function of ETc and ER, and ER is a function of AWHC. High AWHC implies high ER and low water deficit.

The method used in this work has been shown to be efficient in that it takes into account the rainfall intensity and its distribution and the AWHC of the soils of the oil palm area on which water deficit depends. Furthermore, the relative similarity between the values of water deficit in N'gusti (West Cameroon) calculated using a previous work and the one issued from the current method validates the method of prediction of water deficit reported in this paper, suggesting that the annual water deficit as estimated by this method can be used in the yield prediction using one of the equations developed by IRHO (1977), Caliman (1992), and Bakoumé (2011) for Africa and the equation developed by Corley (1996) for South-east Asia. In the above mentioned equations, oil palm yield is seen as a function of water deficit in view of the high correlation between water deficit and fresh fruit bunch yield. Bakoume's (2011) formula was developed for oil palm planting materials of the second cycle of recurrent reciprocal selection. The maximum African annual yield of fresh fruit bunches under satisfactory soil moisture throughout the year is 28 t/ha. Estimated yield in N'gusti using the formula described by Bakoumé (2011) and the water deficit derived from, on the one hand, the modified Penman equation and, on the other hand, the method reported in this paper are 25.9 t/ha and 25.7 t/ha, respectively. Finally, the method here reported provides reliable estimates of the irrigation component of the day-to-day water balance defined by Prioux (1989) as follows: Initial reserves + Rain + Irrigation + ETc = Provision of water for growth and production of oil palm. Water deficits would provide good estimates of water needed for irrigation for optimum yield. This requirement is well supported by the reported method of prediction of water deficit, which takes into account both the initial reserves and the water deficit.

The method has revealed the likelihood of water deficits in months receiving up to 180 mm. Generally, in oil palm, a dry month is considered as a month with less than 100 mm of rainfall. In Malaysian oil palm areas studied, the number of months with water deficit is 9 on average. The wide spread of water deficit throughout the year could result in yield depression even if the values of the water deficit are relatively low (< 50 mm). Failure to accommodate water deficits could explain the gap between targeted yield of 30 t/ha (Corley, 1996) in the 1990s and the lower yields obtained by the plantations despite good agricultural practices. The author has predicted a yield decline of 2.88 t/ha of fresh fruit bunches per 100 mm of water deficit in southeast-Asia. Given the limited source of natural water for irrigation, it is likely that research should opt for the selection of progenies tolerant to drought among the wide range of available planting material from different genetic backgrounds.

4. Conclusion

Water deficits as calculated in this work were close to those obtainable using the Penman equation for the determination of evapotranspiration from an oil palm area. The originality of the method resides in the fact that, not only does it operate with in fact very little climactic data, but it also takes into account the available water-holding capacity of the soil in the estimation of the effective rainfall. Furthermore, the water deficit is not a direct function of the gross rainfall but of the efficient rainfall, which is a product of the available water holding capacity of the soils, rainfall intensity and rainfall distribution. More reliable values of monthly water deficit and monthly water reserves are generated which allow planning for water demands for irrigation of oil palms during periods of soil moisture deficit for enhanced growth and increased yields. Given the fact that water is used for many purposes and also source of conflicts between irrigated agriculture with other nonagricultural users of same water source, it is necessary for needs of water for irrigation to be as accurate as possible and very close to the needs for oil palm growth and production of fresh fruit bunches. Hence, accurate predictions of water deficit would prevent water wastage by excessive irrigation as well as avoid insufficient watering. The wide spread of water deficit in oil palm areas in Malaysia and the failure to accommodate to it may be one of the factors for the gaps between expected and recorded yields of the oil palm industry, even under best agricultural practices.

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Chemical Composition of Four Cultivated Tropical Bamboo in Genus *Gigantochloa*

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Abstract

The chemical compositions of cultivated 3 year-old bamboo culms of *Gigantochloa brang, G. levis*, *G. scortechinii* and *G. wrayi* were studied. The culms exhibited different chemical composition in extractives, α-cellulose, lignin and ash contents between the bamboo species, location in the culms and position at the nodes and internodes. The extractive content in four species ranged from 8.30 to 9.23%. The extractive content of *G.brang, G.levis, G.scortechinii* and *G.wrayi* were 8.30%, 9:23%, 8:00% and 8.62% respectively. The holocellulose content for *G. levis* were 85.08%, *G. wrayi* 84.53%, *G. brang* 79.94% and *G. scortechinii* 74.62%. The holocellulose content for the cultivated bamboo genus *Gigantochloa* were 74% to 85%. The α- cellulose is the chemical constituents in the holocellulose. The highest was *G.brang* (51.58%) followed by *G. scortechinii* (46.87%), *G. wrayi* (37.66%) and *G. levis* (33.80%). The lignin content ranged between 24.84 to 32.65%. The highest were obtained in *G. scortechinii* (32.55%), *G. wrayi* (30.04%), *G. levis* (26.50%) and lowest in *G. brang* (24.83%). The ash content in four species of *Gigantochloa* bamboo ranged between 0.88 to 2.86%. The ash content is the highest in *G. scortechinii* (2.83%) follow by *G. levis* (1.29%), *G. brang* (1.25%) and the lowest in *G. wrayi* (0.88%).

Keywords: bamboo species, holocellulose, hemicellulose, cellulose, lignin, extractives, ash contents

1. Introduction

The chemical contents of the bamboo cell walls consisting of cellulose, hemicelluloses and lignin. These materials are composed of large molecules and constitute 90-98% of the cell wall. The remaining 2 to 10% composed of lower molecular weight compound called extractives. The main constituents of bamboo culms are holocellulose (60-70%), pentosans (20-25%), hemicelluloses and lignin (each amounted to about 20-30%) and minor constituents like resins, tannins, waxes and inorganic salts (Tomalang et al., 1980). The amount of each component, especially holocellulose, lignin and extractive, varies considerably between the materials. Variation in the chemical constituents occurred in different species, location of cell within the tree (Thomas, 1977; Browing, 1975).

The anatomical, chemical and physical-mechanical properties of bamboo exhibit no basic differences among genera and species compare to wood. Also, growth conditions and aging have apparently no significant effect on composition and structure of the bamboo tissue. In brief, the total culm comprises of about 60% parenchyma, 40% fibers and 10% conducting tissue (vessels and sieve tubes) (Razak et al., 2009; Liese, 1992, 1985)

There exist relationships between the chemical composition and utilization, as bamboo consists of about 50-70% holocellulose, 30% pentosans and 20-25% lignin. There are some differences in these main constituents between species, but any influence on technological properties remains uncertain (Liese, 1992). Cellulose and hemicelluloses are carbohydrate polymers constituents of simple sugars monomers, and lignin is a polymer of phenylpropane units (Browing, 1975). Cellulose is long-chain polymer of glucose which differs from starch in configuration. The fibrous nature of the wood cells is the result of linear, oriented, crystalline arrangement of cellulose component. Hemicelluloses are shorter, or "branched polymers of five-carbon sugars (pentoses), such as xylose, or six-carbon sugars (hexoses) other than glucose. They are amorphous in nature and serve with the lignin to form the matrix, in which the cellulose fibrils are embedded. Although the cellulose structure is the same in different species, the hemicelluloses vary considerably among species and especially between hardwoods and soft-woods. Hardwood hemicelluloses are generally richer in pentoses while softwood hemicelluloses generally contain more hexoses.

Lignin, the third cell wall component, is a three-dimensional polymer formed from phenylpropane units which have randomly grown into a complicated large molecule with many different kinds of linkages between the building blocks. Lignin acts as cement between the wood fibres and as a stiffening agent within the fibers. In the production of chemical wood pulps, it is dissolved by various chemical processes, leaving the cellulose and hemicelluloses behind in fibrous form. Some hemicelluloses are lost in the process because of their lower molecular weight, greater solubility and easier hydrolysis.

Chemical composition of cultivated bamboo under genus *Gigantochloa* (*Gigantochloa brang, G. levis, G. scortechinii* and *G. wrayi*) were studied to determine the percentage contents of extractives, α-cellulose, lignin and ash contents between them. A comprehensive knowledge of the chemical components in the bamboo species will facilitate the use of the materials in the industrial forestry sector and help to enhance their utilization in the chemical and bio-chemical industrial.

2. Materials and Method

2.1 Raw Material Preparation

Four (4) species of bamboo namely *G. levis, G. scortechinii* and *G. wrayi* were used in this study. The chemical composition of bamboo was determined following the standard outlined in the TAPPI test method T257 (Anon. 1993). Bamboo samples were divided according to species. Every species were divided according to location of the sample which is node and inter node and also, on the basis of position which is outer, middle and inner layer of the bamboo culms. Samples were chipped and dried in an oven at 50°C for three (3) days. The dried sample were ground into powder with Willey mill in order to pass BS 40-mesh (425 µm) sieve and retained on BS 60-mesh (250 µm) sieved. The size of the sample must be small to make sure the reaction reagent and fibres are occurring with optimum during the analysis. The chemical composition analysis were used on the bamboo fibre. This method were divided into five stages namely, the material preparation, determination of extractive, holocellulose, cellulose and lignin content.

2.2 Determination of Organic and Ash

The major chemical constituents of bamboo from genus *Gigantochloa* were determined. These include holocellulose, lignin, α-cellulose and extractives. The chemical characteristics of bamboo were determined in accordance with the standards outlined in TAPPI test methods. The determination of extractive content were carried out in according to the T204 cm-88 and T264 cm-88 methods (Anon., 1999). The procedure for the ethanol-benzene solubility followed T204 cm-97 (Anon., 2002) for 6 h. Holocellulose were determined following the procedure of Le Wise et al. (1946) and α-cellulose base by T203 cm-74 (Anon., 1999). The lignin content were carried out following the T222 cm-88 method (Anon., 2002). The inorganic constituent of lignocellulosic material (referred to its ash content) was considered being the residue remaining after combustion of the organic matter at a temperature of 525±25°C. The ash content was determined by followed the procedure outlined in T211 cm-93 method (Anon., 1999).

3. Results and Discussion

3.1 Extractive Content

Table 1 shows the results of the extractive content for various bamboo species at different location (internodes and nodes) and different position (inner, middle and outer layer of the culm). The extractive content in four species of *Gigantochloa* bamboo ranged between 8.30 to 9.23%. Norul Hisham et al. (2006) obtained the extractive content of *G.scortechinii* from 3.4 to 5.8%. Mahanim et al. (2008) on their study on *G.scotechinii* and *G. lagulata* obtained the extractive content ranging from 3.74 to 4.45% and 2.95 to 3.20% respectively. The result obtain from this study was higher compared with previous studies. Softwoods constitute 3% and the hardwoods 5% extractives (Thomas, 1977). The extractive content of *G. brang, G. levis, G. scortechinii* and *G. wrayi* were 8.30%, 9:23%, 8:00% and 8.62% respectively (Table 1). The extractive may constituted roughly from 5% to 30% of wood substance in wood, depending on factors such as species, growth conditions, and time of year when the tree were cut.

Results shows that the extractive content in bamboo were less than 10% and were higher than those found in softwoods and hardwoods. The analysis of Variance (ANOVA) on extractive content (Table 6) showed significant differences on extractive content within the various bamboo culms. The extractive content were noted to be higher in *G. levis* (9.23%), followed *G. wrayi* (8.62%), *G. brang* (8.30%) and lowest in *G. scortechinii* (8%). At the internodes, the extractive content was 8.46%, while at nodes was 8.63% for all species. This showed a significant difference between the internodes and nodes in the bamboo culm.

Both the internodes and the nodes have higher extractives content in the inner layers compared to the outer layers of the bamboo. The inner layers of the internodes contains about 12.32% to 14.17% and the nodes at 11.90 to 16.41%, compared with *Phyllostachys pubescens* at inner layer at 5.78% (Yoshizawa et al., 1991). Middle position for the internodes was from 5.15 to 9.74% and for nodes at 5.71 to 7.57 % Compared to *P. pubescens* at middle layer was 4.25%. For the outer layers for internodes was from 3.65 to 4.75% and for nodes was 4.71 to 6.66%, compared with *P. pubescens* at outer layer was 3.15%. The extractive contain for different layers shows significant differences between the bamboo position for all species (Table 6). The highest extractive content was at 13.42 % inner layer, 7.21% at the middle layer and 4.99% at outer layer. A similar trend of extractive content at inner, middle and outer position in bamboo culm was reported by Li et al. (2007). It showed that the extractive composition between sympodial and monopodial type of bamboo exhibit the similar trend. Li et al. (2007) noted that the inner part has higher extractive content due to wax like materials attached to the inner layer and may have contributed to higher extractive content, compared with the middle and outer layer.

Table 1. Average extractive content various species Genus *Gigantochloa*

Sample Location	Position	***G. brang***	***G. levis***	***G. scortechinii***	***G. wrayi***
	Outer	4.42 (±0.34)	4.76(±0.38)	3.62 (±0.16)	4.54 (±0.47)
Internode	Middle	5.16(±0.05)	9.89(±0.27)	6.58(±0.28)	9.57 (±0.19)
	Inner	12.32(±0.24)	14.03(±0.40)	14.17(±0.18)	12.40(±0.48)
	Average	7.30(±0.21)	9.53(±0.35)	8.16(±0.21)	8.80 (±0.38)
	Outer	5.25(±0.04)	5.90(±0.80)	4.72(±0.30)	6.66 (±1.19)
Node	Middle	6.27(±0.42)	7.53(±0.47)	7.01(±0.15)	5.71(±0.88)
	Inner	16.41(±0.25)	13.27(±0.20)	11.91(±0.18)	12.88(±2.61)
	Average	9.31(±0.24)	8.93(±0.49)	7.80(±0.21)	8.41(±1.56)
	Total average	8.30(±0.23)	9.23(±0.42)	8.00(±0.21)	8.62(±0.97)

The data presented in this report are average values of at least 5 samples.

3.2 Holocellulose Content

The result of the chemical analysis shown in Table 3 indicated only a small difference exist in the holocellulose content between the bamboo species. The highest was *G. levis* (85.08%) followed by *G. wrayi* (84.53%), *G.brang* (79.94%) and *G. scortechinii* (74.62%) respectively. Based on this result, the mean average of holocellulose content for the cultivated bamboo genus *Gigantochloa* were 74% to 85%. Tamalong et al. (1980) and Chen et al. (1985) reported that the holocellulose content in bamboo normally consists about 50-70%. The analysis of variance (ANOVA) on holocellulose are tabulated in Table 6 showed that there exist significant different between species. The highest holocellullose contents was 84.53% found in *G. wrayi* and 84.52% in *G. levis*, *G. brang* 79.70% and *G. scortechinii* has the lowest value at 74.62%. An earlier studies by Norul Hisham et al. (2006) found the holocellulose content of *G. scortechinii* was in the range of 78.60 to 82.30%. Ireana (2010) reported that the holocellulose content in *B. blumeana* was 74.56 %. Li et al. (2007) on their study on *P. pubescens* bamboo showed that the holocellulose content was 63.14 to 69.94%. The value of holocellulose content in bamboo fiber was higher compared to holocellulose content in *P. pubescens* bamboo and almost similar to softwood fiber (60-80%) (Tsoumis, 1991; Ashori, 2006).

The holocellulose content in bamboo ranged from 74-85% and is higher than softwood (67%) and also similar and higher then hardwood (75%). The holocellulose content for *G.levis* was the higher (85.08%) followed by *G. wrayi* (84.53%), *G. brang* (79.94%) and *G. scortechinii* (74.62%). The holocellulose content does not show much significant differences at the internodes and nodes of the bamboo culms between the various bamboo species. The content of holocellulose at internodes was 73.48% to 86.74% and for the nodes 76.76% to 85.65%. The holocellulose content were higher at the outer position, and its lower in inner position of the bamboo culms (Table 2).

Outer layer for the internodes possess about 75.36 % to 91.13% and for the nodes was 77.80 to 88.55% of holocellulose, compared with *Phyllostachys pubescens* at outer layer 69.94%, middle position for interrnodes were from 71.40 to 84.92% and the nodes at 77.26 to 88.29%. For the inner layer of internode were 70.69 to 84.17% and at node was 75.23 to 81.97%. At the inner layer the holocellulose content were 64.54%, the outer layer has 82.99%, middle layer 80.89% and the inner layer 78.65%.

Similar trend of holocellulose content at inner, middle and outer positon in the monopodial type of bamboo culm was observed by Li et al. (2007). This shows that the holocellulose composition between sympodial and monopodial bamboo exhibit similar trend. The outer layer possess higher holocellulose content due to heavy distribution of vascular bundle compared to the inner layers.

Table 2. Average holocellulose content of Genus *Gigantochloa*

Sample Location	Position	***G. brang***	***G. levis***	***G. scortechinii***	***G. wrayi***
	Outer	80.05(± 0.93)	89.80(± 0.92)	75.35(±0.21)	84.49(±1.45)
Internode	Middle	78.29(± 0.48)	83.83(±2.55)	71.41(± 0.45)	82.75(±0.76)
	Inner	76.64(±0.34)	84.08(±0.68)	70.67(±0.27)	82.98 (±1.34)
	Outer	83.72(± 0.55)	84.13(±0.81)	77.84(±0.28)	88.55 (±0.77)
Node	Middle	81.97(±1.00)	83.33(±0.21)	77.26(±0.41)	88.28 (±1.98)
	Inner	77.53(± 0.51)	81.97(±0.71)	75.24(±0.29)	80.11 (±1.84)

The data presented in this report are average values of at least 6 samples.

3.3 α- cellulose Content

The analysis of variance (ANOVA) on α-cellulose content is showed in Table 6. Significant difference were noted in the α-cellulose content between the various bamboo species. The highest α-cellulose content were observed in species *G. brang* (51.58%) followed by *G. scortechinii* (46.87%), *G.wrayi* (37.66%) and *G. levis* (33.80%). The α-cellulose is one of the chemical constituents in the holocellulose. The α-cellulose content for the cultivated bamboo genus *Gigantochloa* ranged from 33.79 to 51.76%. A similar pattern was obtained by Mahanim et al. (2008) reported that the α-cellulose contents in bamboo *G.scortechinii* and *G. lagulata* consists about 46.14-46.53% and 48.4-56.45% respectively. Other studies on the α-cellulose content of *G. scortechinii* was in the range of 63.30 to 64.60% (Norul Hisham et al., 2006). Ireana (2010) reported that the α-cellulose content in bamboo (*Bambusa blumeana*) consist of 58.72 %. Li et al. (2007) on their study on *P. pubescens* bamboo showed that the α- ellulose content was 41.71 to 49.02%. The value of α-cellulose content in bamboo fiber studied was similar compared to α-cellulose content in *P. pubescens* bamboo and almost similar to softwood fiber (42%) (Thomas, 1977). The α-cellulose content in bamboo (33.79-51.76%) were higher than softwood (42%) and hardwood (45%).

The α-cellulose content at difference location internode and node in bamboo culm for this genus, did not shows much difference between internodes and nodes. The content of α- cellulose at internode was 31.76% to 50.82% and for the node 33.79% to 51.76%.

Table 3. Average α-cellulose content Genus *Gigantochloa*

Sample Location	Position	***G. brang***	***G. levis***	***G. scortechinii***	***G. wrayi***
	Outer	56.94(±0.41)	36.96(±0.54)	61.31(±0.43)	44.20(± 0.90)
Internode	Middle	48.98(±0.27)	32.87(±1.25)	41.59(±0.73)	38.10(±1.42)
	Inner	46.34(±0.63)	25.54(±0.52)	39.16(±0.49)	34.62(±1.37)
	Outer	57.98(±0.79)	46.46(±0.68)	50.02(±0.76)	38.72(±2.39)
Node	Middle	52.71(±1.06)	32.02(±0.20)	46.18(±0.48)	37.80(±2.70)
	Inner	46.54(±0.43)	29.00(± 0.47)	42.95(±0.23)	32.55(±1.40)

The data presented in this report are average values of at least 6 samples.

The ANOVA in Table 6 shows no significant difference in α-cellulose content between the internodes and nodes. The value of α- cellulose content at internodes was 42.22% and for the nodes was 42.74%.

The outer position for the internodes contains 36.99% to 61.24% and for the nodes at 38.74 to 58.15%, compared with *P. pubescens* (Moso bamboo) which has 49.02% at outer layer (Yoshizawa et al., 1991). At the middle position the α- cellulose content for internode was from 32.72 to 49.08% and for node was 32.06 to 53.33%, compared with *P. pubescens* at middle layer was. 45.08%. The inner position, the α-cellulose content for internodes was from 25.56 to 46.32% and for the nodes was 28.95 to 46.61%, compared to 42.84%. in inner layer of *P. pubescens*.

3.4 Lignin Content

The Klason lignin or acid insoluble lignin values are shown in Table 4. The results show the lignin content of various bamboo species under genus *Gigantochloa* at difference location (internode and node) and difference position (inner, middle and outer layer) of the culm. The lignin content in four species of *Gigantochloa* bamboo is in the ranged of 24.84 to 32.65%. Norul Hisham at el. (2006) Previous obtained lignin content of *G.scortechinii* at range of 23.40 to 29.00%. Mahanim et al. (2008) on their study on *G. scotechinii* observed that the lignin content was 16.12-12.48% and *G. lagulata* 12.02-11.69%. Ireana (2010) reported that the lignin content in bamboo (*B. blumeana*) consist of 28.86 %. Data obtains from Ireana (2010) study almost comparable with result from this study. Lignin constitute 23% to 33% of wood substance in softwood and 16% to 25% in hardwoods, Although lignin occurs in wood throughout the cell wall, it is concentrated toward the outside of the cells and between cells.

The analysis of variance (ANOVA) on lignin contents (Table 6) shows significant differences between the bamboo species. The results showed the highest value in *G. scortechinii* i.e. 32.55%, *G. wrayi* i.e. 30.04%, *G. levis* i.e. 26.50% and lowest value in *G. brang* i.e. 24.83%. The lignin contents in bamboo is almost similar with that of the softwood and hardwood. The lignin content at internodes was 15.72% to 43.68%, while at nodes was 18.28% to 33.33% for all species. The lignin content at the internode was 32.19% and at the node was 24.76%. There was a significant different between location in bamboo culm. It's clear that internode content higher lignin amount compare to node.

The inner position for the internode contains about 29.88% to 35.36% and for the node at 19.66 to 29.93%.In comparison with *P. pubescens* (Moso bamboo) at inner layer of internodes was 22.57%, the middle layer at 15.70 to 39.40% and for the nodes was 18.28 to 27.74 %, the middle layer was 21.79% (Yoshizawa et al., 1991). The outer position for the internodes was from 35.98 to 43.68% and for the nodes was 21.01 to 33.33%, compared with *P. pubescens* at outer layer was 24.30%. The analysis of variance showed there was a significant difference of lignin content between positions of bamboo culm thickness. The value was highest at outer-layer which was 33.43%, inner layer 30.03% and the lowest at middle layer which was 21.98%. Similar trend were obtained by Li et al. (2007) on lignin content at inner, middle and outer positon in bamboo culm. There was a clear relationship between the lignin content with the fiber length. The middle position has a long fibre. The outer and inner positions having short fibres possess higher lignin content. Lignin is often called the cementing agent that binds individual cells together (Miller et al., 1999).

3.5 Ash Content

Table 5 showed the ash content for various bamboo species under genus *Gigantochloa* at difference location (internodes and nodes) and difference position which is inner, middle and outer layer of the culm. As it is shown in the table, the mean average of the ash content in four species of *Gigantochloa* bamboo, which are in the range of 0.88 to 2.86%. Norul Hisham et al. (2006) found that the ash content of *G. scortechinii* was the ranged of 1.90 to 3.50%. Ireana (2010) reported that the ash content in *B. blumeana* consist of 1.67%. Both results obtained are comparable with the result obtained from this study.

Table 4. Average lignin content of Genus *Gigantochloa*

Sample Location	Position	***G. brang***	***G. levis***	***G. scortechinii***	***G. wrayi***
	Outer	38.75(±0.45)	35.98(±0.42)	43.68(±0.47)	38.90(±0.20)
Internode	Middle	15.72(± 0.41)	20.34(±0.98)	28.68(±0.40)	22.39(±0.44)
	Inner	35.60(±0.66)	33.32(±0.43)	33.57(±0.22)	39.40(±1.12)
	Outer	21.01(±0.66)	25.42(±0.84)	33.33(±0.19)	30.34(±2.43)
Node	Middle	18.28(±0.53)	21.67(±0.37)	27.61(±1.04)	21.04(±0.83)
	Inner	19.69(±0.62)	22.30(±0.10)	28.42(±1.04)	28.15(±3.80)

The data presented in this report are average values of at least 6 samples.The data presented in this report are average values of at least 6 samples.

The highest value for *G. scortechinii* was 2.83% follow by *G. levis* 1.29%, *G. brang* 1.25% and the lowest value for *G.wrayi* 0.88%. Several common wood species have ash contents ranging from 0.43% (aspen) to 0.87% (white oak) (Misra et. al. 2004). The bamboo genera *Gigantochloa* had significantly higher ash content than these common wood species, but similar with kenaf form 1.6 -22 % (Ashori, 2006).

The inorganic components of wood are generally expressed as percentage of ash, based on dry weight of the sample. The normal levels of occurrence appear to be from approximately 0.1% to about 0.5% ash, for most domestic timbers. Scurlock et al. (2000) reported that *Phyllostachys nigra, P. bambusoides, P. bissetii* bamboo species from 1 to 4.5 years old have ash content 0.41 to 0.87%, 0.53 to 0.84%, and 0.78 to 1.14% respectively.

The results in Table 5 showed the ash contents at internodes was 1.00% to 2.32%, while at nodes was 0.88% to 2.86% for all species. There is no significant difference of ash content between the internodes and nodes in the culm.

The ash contents at the inner position for the internode were about 1.38% to 3.39% and for the node was 0.53 to 4.79%, compared with *Phyllostachys pubescens* (Moso bamboo) at inner layer was 0.88%, At the middle layer position for the interrnode was from 0.86 to 2.03% and for the node was 0.64 to 2.62 % compared with *P. pubescens* at middle layer was 0.65%. For the outer layer position for the internode was from 0.78 to 1.53% and for the node was 0.71 to 2.80%, compared with *P. pubescens* at outer layer was 0.54%. Li et al. (2007) from their study showed the similar trend of ash content at inner, middle and outer positon in bamboo culm. It's showed that the ash composition between sympodial and monopodial type of bamboo, has a similar trend.

Table 5. Average Ash content Genus *Gigantochloa*

Sample Location	Position	***G. brang***	***G. levis***	***G. scortechinii***	***G. wrayi***
	Outer	0.78 (±0.04)	1.19 (±0.14)	1.50 (±0.11)	0.97 (±0.12)
Internode	Middle	0.84(±0.06)	1.26 (±0.18)	2.04 (±0.06)	0.97 (±0.13)
	Inner	1.38 (±0.12)	1.41 (± 0.11)	3.35 (± 0.23)	1.41 (± 0.18)
	Outer	1.26 (±0.24)	1.66 (± 0.26)	2.80(±0.14)	0.72 (± 0.14)
Node	Middle	0.83 (±0.06)	1.03 (± 0.02)	2.63(±0.11)	0.66 (± 0.12)
	Inner	1.13(±0.11)	1.24(± 0.15)	4.70 (±0.28)	0.53(± 0.24)

The data presented in this report are average values of at least 6 samples.

3.6 Comparison of Chemical Composition

Table 6. Summary Result for Analysis of Variance (ANOVA) for chemical composition between species, location and position

	Chemical Composition				
	Extractive	Holocellulose	Alpha Cellulose	Lignin	Ash
SPECIES					
G.brang	8.30b	79.70b	51.58a	24.83c	1.26cb
G.levis	9.23a	84.52a	33.81d	26.50c	1.30b
G.scortechinii	8.00b	74.63c	46.87b	32.55a	2.84a
G.wrayi	8.62ab	84.53a	37.66c	30.04b	0.88c
LOCATION					
Internode	8.46b	80.03b	42.22a	32.19a	1.54b
Node	8.63a	81.66a	42.74a	24.76b	1.60a
POSITION					
Outer layer	4.99c	82.99a	49.07a	33.43a	1.52b
Middle layer	7.21b	80.89b	41.28b	21.98c	1.28c
Inner layer	13.42a	78.65c	37.09c	30.03b	1.89a

Means followed by the same letter in a column is not significant different at 0.05 probability level.

3.7 Comparison between Chemical Composition with Moisture Content and Specific Gravity

Moisture contents were proportional to the extractive content. The higher the moisture content, the higher the extractive content. Lignin content does not show significantly correlation with moisture content. The correlation was rather weak and the moisture content has no direct relationship with lignin content in bamboo. Holocellulose and alpha-cellulose content has significant correlation with moisture content. They correlate quite well where the location and position with high moisture content, it related directly with low contents of holocellulose and alpha-cellulose.

The ash content has a positive correlation with moisture content. This means that the location and position that contains a high moisture content, ash directly a rate that is higher. Water contents were most likely contained minerals or inorganic materials. Ash was an indicator of the presence inorganic materials in the lignocellulose material.

Table 7 shows correlation between chemical compositions with specific gravity. Extractive content has a significant correlation with specific gravity of bamboo. This shows the inverse relationship which a higher specific gravity, extractive content was low.

Lignin content has a significant correlation with specific gravity. This means that the location and position has a high specific gravity, the lignin content was relatively high. It shows the role of lignin as cement for cell bonding. This clearly shows a weak correlation between specific gravity and lignin content.

Holocellulose and alpha-cellulose has a significant correlation with specific gravity. This shows a strong and positively relationship, meaning a high specific gravity, which was attributed to the presence of a high holocellulose and alpha-cellulose. The ash content with specific gravity relationship is not consistent and not significant.

Table 7. Correlation coefficients of different chemical composition with bamboo in genus *Gigantochloa* moisture content and specific gravity

Properties	Species	MC	SG
Extractive	*G. brang*	0.69*	-0.77*
	G. levis	0.75*	-0.83*
	G. scortechinii	0.84*	-0.83*
	G. wrayi	0.50*	-0.73*
Lignin	*G. brang*	0.09ns	0.24*
	G. levis	0.01ns	0.18*
	G. scortechinii	0.09ns	0.38*
	G. wrayi	0.09ns	0.22*
Holocellulose	*G. brang*	-0.77*	0.79*
	G. levis	-0.34*	0.48*
	G. scortechinii	-0.61*	0.49*
	G. wrayi	-0.39*	0.56*
Alpha-cellulose	*G. brang*	-0.83*	0.94*
	G. levis	-0.71*	0.81*
	G. scortechinii	-0.70*	0.78*
	G. wrayi	-0.43*	0.74*
Ash	*G. brang*	0.35*	-0.16*
	G. levis	0.20*	-0.22*
	G. scortechinii	0.38*	-0.24*
	G. wrayi	0.25*	-0.08ns

MC=moisture content, SG= specific gravity, ns= not significant, * = significant at 0.05, * =significant at 0.05 probability level.

3.8 Comparison between Chemical Composition with Anatomy and Fiber Morphology

Table 8 shows that the extractive content has a clear relationship with the number of vascular bundle. This means that the number of vascular bundle increases, depleted extractive content. This means that the larger size of the vascular bundle, the higher the extractive content in bamboo.

Lignin content had a significant correlation with the number of vascular bundle. This indicates heavy distribution of the vascular budle. The was a weak correlation between vascular bundle size (length and width) with lignin content.

The correlation between fiber morphology (Table 8) shows no relationship exist between lignin content and the fiber fiber length, but there was a clear correlation on the lignin content and fiber diameter. Lignin content increases when the fiber diameter were smaller, this confirms the presence of lignin as binder to bind the fibers. Small diameter fibers need a lot of binder (lignin) to form a combined cell structure of bamboo.

The lumen diameter has a negative relationship with lignin content. Lignin content had a significant correlation with the wall thickness. This means the fiber has a thin wall thickness has higher lignin content at that area.

Table 7 shows the holocellulose contents and alphacellulose was positively correlate with the number of vascular bundle. Instead, it has a negative relationship with the vascular bundle size (length and width). Holocellulose and alphacellulose content was not showing a clear relationship with the fiber morphology properties. Similarly, ash content of the relationship, it was also not clear.

Table 8. Correlation coefficients of different chemical composition with anatomical and fibre properties of various bamboo species genus *Gigantochloa*

Properties	Species	Anatomical Properties			Fibre Properties			
		No. Vascular Bundle	Vascularbundle length	Vascularbundle width	Fibre length	Fibre diameter	Lumen diameter	Wall thickness
Extractive	*G. brang*	-0.64*	0.68*	0.48*	0.01^{n}	0.09*	0.13*	-0.07^{n}
	G. levis	-0.65*	0.77*	0.65*	0.08^{n}	0.16*	0.09*	-0.15*
	G. scortechinii	-0.84*	0.65*	0.49*	0.06^{n}	0.10*	0.12*	-0.12*
	G. wrayi	-0.37*	0.67*	0.32*	0.01^{n}	0.09^{n}	0.09^{n}	-0.14*
Lignin	*G. brang*	0.35*	0.01^{n}	-0.42*	-0.05^{n}	-0.47*	-0.23*	-0.41*
	G. levis	0.37*	0.11*	-0.23*	-0.03^{n}	-0.35*	-0.23*	-0.31*
	G. scortechinii	0.72*	0.14*	-0.59*	-0.09^{n}	-0.30*	-0.37*	-0.38*
	G. wrayi	0.38*	0.21*	-0.32*	-0.07^{n}	-0.36*	-0.22*	-0.37*
Holo-cellulose	*G. brang*	0.30*	-0.44*	-0.13*	-0.00^{n}	-0.35*	-0.04^{n}	0.23*
	G. levis	0.54*	-0.33*	-0.12*	-0.03^{n}	-0.34*	-0.18*	0.29*
	G. scortechinii	0.40*	-0.13*	-0.06*	-0.02^{n}	-0.35*	-0.13*	0.36*
	G. wrayi	0.31*	-0.43*	-0.16*	-0.05^{n}	-0.34*	-0.07^{n}	0.10*
Alpha-cellulose	*G. brang*	0.65*	-0.66*	-0.40*	-0.30*	-0.08^{n}	-0.21*	-0.02^{n}
	G. levis	0.57*	-0.75*	-0.76*	-0.28*	-0.14*	-0.03^{n}	-0.14*
	G. scortechinii	0.86*	-0.60*	-0.47*	-0.28*	-0.04^{n}	-0.24*	-0.15*
	G. wrayi	0.55*	-0.75*	-0.53*	-0.27*	-0.00^{n}	-0.14*	-0.05^{n}
Ash	*G. brang*	-0.23*	0.27*	-0.03^{n}	-0.18*	-0.08^{n}	-0.08^{n}	-0.14*
	G. levis	-0.12*	-0.05^{n}	-0.07^{n}	-0.02^{n}	-0.11*	-0.03^{n}	-0.10*
	G. scortechinii	-0.22*	0.26*	-0.13*	-0.06^{n}	-0.13*	-0.14*	-0.25*
	G. wrayi	-0.26*	-0.27*	-0.23*	-0.12*	-0.17*	-0.10*	-0.16*

4. Conclusions

The extractive content in four species of *Gigantochloa* bamboo is in the range of 8.30 to 9.23%. The extractive content of *G.brang*, *G. levis, G. scortechinii* and *G. wrayi* are 8.30%, 9:23%, 8:00% and 8.62% respectively. The extractive content was higher in *G. levis* (9.23%), followed *G. wrayi* (8.62%), *G. brang* (8.30%) and lowest value in *G. scortechinii* (8%). The extractive content at internode was 8.46%, while at the node was 8.63% for all species. Inner position for the internodes the extractives contains were 12.32% to 14.17% and for the node were 11.90 to 16.41%. The middle position for internode were from 5.15 to 9.74% and node were 5.71 to 7.57 %. The extractive content was at 13.42 % (inner layer), 7.21% (middle layer) and 4.99% (outer layer).

The holocellulose content for *G. levis* was 85.08%, *G. wrayi* 84.53%, *G. brang* 79.94% and *G. scortechinii* 74.62%. The holocellulose content for the cultivated bamboo genus *Gigantochloa* were 74% to 85%. The content of holocellulose at internode was 73.48% to 86.74% and for the node 76.76% to 85.65%. The holocellulose content is highest at internode (81.65%) compared to node (80.02%).

The highest was *G. brang* (51.58%) followed by *G. scortechinii* (46.87%), *G. wrayi* (37.66%) and *G. levis* (33.80%). Based from the result, the average of α-cellulose content for the cultivated bamboo genus *Gigantochloa* were 33.79 to 51.76%. The α-cellulose content at difference location internode and node in bamboo culm for this genus, showing not much differences between internodes and nodes. The content of α- cellulose at internodes was 31.76% to 50.82% and for the nodes 33.79% to 51.76%. The average value of α- cellulose content at internodes was. 42.22% and for the nodes was 42.74%.

The lignin content in the four species of *Gigantochloa* bamboo ranged between 24.84 to 32.65%. The highest were obtained in *G. scortechinii* (32.55%), *G. wrayi* (30.04%), *G. levis* (26.50%) and lowest in *G. brang* (24.83%). The lignin content at the internodes were 15.72% to 43.68%, while at nodes were 18.28% to 33.33% for all species.

The ash content in four species of *Gigantochloa* bamboo ranged between 0.88 to 2.86%. The ash content is the highest in *G. scortechinii* (2.83%) follow by *G. levis* (1.29%), *G. brang* (1.25%) and the lowest in *G.wrayi* (0.88%). The ash content at internodes ranged from 1.00% to 2.32%, while at nodes was 0.88% to 2.86% for all species. The ash content were higher at nodes compared to internodes. The ash contents at the inner position for the internodes ranged between 1.38% to 3.39% and the nodes was 0.53 to 4.79.ListenRead phonetically

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Analysis of Constraints of Rural Beef Cattle Cooperative Farmers: A Case Study of Ga-kibi, Norma and Mogalakwena in Blouberg

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Abstract

Agricultural co-operatives are tools used to accelerate the process of agricultural development. Co-operatives in South Africa are useful tools used to assuage poverty and improve the living standard of the rural household through pooling together the available resources. There is emerging evidence that beef cooperatives and their members can benefit from market oriented agriculture when smallholders farmers are integrated into the value chain management. Furthermore, institutional provisions along the value chain and policies seldom prioritize the needs of smallholder farmers and thus increased the barriers to production and market access. The purpose of this study is to evaluate production and marketing constraints as perceived by beef cattle cooperative farmers in Ga-kibi, Norma, and Mogalakwena. The specific objectives of the study were: (i) to assess production and marketing constraints of beef farmers' cooperative; and (ii) to determine the role of speculators (traders) in marketing of beef cattle. The paper succinctly examines the tottering problems of speculators and concomitant contribution in the marketing of beef animals in the study areas. The study adduces some reasons for the poor performance of beef co-operatives in achieving their goal. These include amongst others: problem of pest and diseases, water stress, inadequate marketing infrastructures, insufficient market access, price fixing of culled stocks, labour and stock pilfering. Logistic regression was used to evaluate perception of constraints by beef cooperative farmers'. The response pattern revealed that the ability of farmers to perceive constraints increases with number of years of farming experience. The study concludes with proposals to improve the performance of agricultural co-operatives in the study areas.

Keywords: beef co-operatives, speculators, infrastructures, income source, constraints

1. Introduction

1.1 Background of the Study Area

Governments of developing countries have often recognized the use of Farmers' Cooperatives to enhance the performance of small-scale farmers. Since 1994, the new democratic government in South Africa has been supporting the growth of cooperatives, especially among historically deprived South Africans, as a strategy to assuage poverty (Tukuta, 2011). The new Cooperatives Act (No. 14 of 2005) under which a variety of cooperatives emerged, was signed into law in August 2005. This Act recognizes that a sovereign and self-sustaining cooperative undertaking can play a major role in the economic and social improvement of small scale farmers (Government Gazette, 2005). However, the new Act emphasised traditional cooperatives where a group of people pull their resources together to satisfy common economic and social needs. According to Philip (2003), cooperatives have significant prospect in contributing towards poverty reduction, enhancing enablement empowerment and generating employment. However, several factors have hindered the performance of smallholder cooperatives in South Africa. A study conducted by Machethe (1990) on poor-performing and failed cooperatives in the former homelands of South Africa asserted that co-operators had no sufficient insight with respect to the purpose, responsibilities and management. Van der Walt's (2005) in his study on "cooperative failures in Limpopo province observed that poor supervision, lack of training, conflict among members and inadequate funds were identified as important causative factors. Similar studies by Kherallah and Kirsten, (2002) emphasised access to start-up capital; knowledge and training in business administration, marketing and bookkeeping; low-levels of education and assertiveness towards work as the causes of non-performance of cooperatives. In many developing countries, including South Africa cattle industry is one of the largest farming enterprises. Beef farming has a low input arrangement in terms of costs, labour, time, asset and it contributes to rural livelihood. The cooperative approach to beef production was aimed at enhancing the sales and procurement of inputs, with added benefit of improving the bargaining power of beef cattle farmers in the area, particularly in relation to market prices. In addition, the idea was to reduce the influence of the speculators (traders) on prices of livestock. The availability of formal marketing arrangements is essential for real marketing of livestock. These arrangements comprise of marketing agents, marketing information and value-adding activities within the reach of the producers. However, these measures are missing in most cases especially for rural livestock producers (NDA, 1998); resulting in the restriction of marketing opportunities for smallholder farmers. According to (Bienabe et al., 2004) smallholder farmers in rural areas, have little market information epitomized by level of literacy. In most instances, information is gathered through interaction with other actors in the commodity chain, but the accuracy of this information is not guaranteed, since those actors might be displaying "opportunistic behavour" (Bienabe et al., 2004). Farmers often sell their livestock at prices that are below the market price because of lack of knowledge of local, regional and national prevailing prices. It is therefore, imperative to assess cooperative farmers' constraints in the three villages (Ga-kibi, Norma, & Mogalakwena) of BLM.

1.2 Purpose and Objectives

The purpose of this study is to evaluate production constraints as perceived by beef cattle cooperative farmers in Blouberg Local Municipality. The specific objectives of the study were:

(i) To assess production and marketing constraints of beef farmers' cooperative;

(ii) To determine the role of speculators (traders) in marketing of beef cattle.

2. Materials and Method

2.1 Description of the Study Area

The study took place in Blouberg Local Municipality (BLM) area which is located in the far northern part of the Capricorn district. Aganang surrounds Blouberg on the South, Molemole on the South West, Makhado on the North East, Lephalale on the North West, Mogalakwena on the Southwest and Musina on the North. The Blouberg Local Municipal area covers an area of approximately 5054 km^2 from Polokwane (Appendix 1). The BLM area are inhabited by Northern Sotho (Pedi tribe) with an estimated population of 194 119. (Statistics, South Africa 2007). There are 14 clusters (community groupings) within Blouberg Local Municipality but only three (Ga-Kibi, Mogalakwena, & Norma) were identified for the study (Appendix 1). The Blouberg local Municipality receives an annual precipitation of about 380 and 550 mm, which falls mostly during summer months (December-January) with average annual temperature of 14-20°C. The Blouberg Local Municipality (BLM) has also a mixture of sweet and sour grass in some parts of the area but mainly, the area is covered with sweet veld. The grasses that are most common are annual grasses that quickly disappear in winter thereby leaving the ground bare (Zwane, 2006).

2.2 Sample and Sampling Procedure

Total of 89 beef cooperative farmers consisting of male and females that were involved in livestock production on sustenance and commercial purposes in Blouberg Local Municipality were purposely selected for this study. In sum, 47 samples of respondents were taken from Ga-kibi, 17 from Norma and 25 from Mogalakwena. A well-structured questionnaire to assess the production and marketing constraints, and the role of speculators in marketing of beef cattle in the study areas was designed in August, 2012. After a pre survey test on beef cooperative farmers and speculators, the questionnaire was modified with the inclusion of few questions.

2.3 The Approach

A co-operative is an independent association of persons integrated willingly to meet their common economic, social and cultural needs through a jointly owned enterprise operated on co-operative principles**.** Speculators are branded traders noted by having access to land and pastures which enable them buy animals at low prices and convert these animals into market-ready animals for resale. A well-structured questionnaire to assess the production and marketing constraints, and the role of speculators in marketing of beef cattle in the study areas was designed in August, 2012. After a pre survey test on beef cooperative farmers and speculators, the questionnaire was modified with the inclusion of few questions. Respondents were interviewed using English and/or Sepedi for farmers who could not speak English. A focus group discussion was also held with the cooperatives farmers. The scheduled focus group meeting was facilitated by the Extension officer attached to the Department of Agriculture in Blouberg Local Municipality. The meeting for the focus group discussion was pre-arranged to coincide with the beef cooperative farmers' normal meeting day. However, prior to the focus group discussion veld drive was carried out with the selected farmers to identify the available production and marketing resources in these areas. The speculators/traders were interviewed telephonically on a pre-arranged date and time to elicit information from them on price fixing procedure. The respondents' demographic information, farm characteristics, beef cattle production and marketing constraints, and the role of speculators in marketing of culled beef cattle were recorded. For each question, the proportion of farmers who gave the same responses was calculated for each village and the percentages calculated based on the total number of farmers who responded to each question. Cooperative farmers who failed to respond to certain question were sponged in the analysis. The data collected was reviewed for errors and were pre-set, entered into Microsoft Excel 2010 spread sheet for analysis. The data were analysed using SPSS and statistical Analysis System (SAS) 2003.

3. Results and Discussion

3.1 Demographic Characteristics of Farmers' in the Ga-kibi, Norma and Mogalakwena

The summary of the demographic data provided in Table 1 portrays that 67% (Ga-kibi), 24.1% (Norma) and 31% (Mogalakwena) of the beef cooperative farmers were males. In contrast female membership of beef cooperatives stood at 34% Ga-kibi, 9.7% Norma and 22.6% Mogalakwena respectively. The ages between 51-60 years, that is 62.5% for Ga-kibi 16.7% Norma and 20.8% for Mogalakwena were involved with beef cooperative (Table 1). The result also shows that the ages between 30- 40years are less inclined to joining farmer's cooperatives as illustrated in Table 1. Overall, respondents in the three areas (Ga-kibi 62.5%, Norma 12.5% and Mogalakwena 25%) had no formal education, while 50% in Ga-kibi, 14.3% in Norma and 35.7% of the beef cooperative had high school education. However, the respondents that had primary education (Table 1) were 58.8% in Ga-kibi, 23.5% in Norma and 66.7% in Mogalakwena.

Table 1. Descriptive statistics for demographic characteristics of farmers in the study areas

		VILLAGES		
		Gaki-bi (N=47) %	**Norma (N=17) %**	**Mogalakwena (N=25) %**
Gender	Male	67.7	24.1	31.0
	Female	37.3	9.7	22.6
Age	<30 years	40.0	20.0	40.0
	30 – 40	50.0	20.0	30.0
	41- 50	36.8	21.1	42.1
	51- 60	62.5	16.7	20.8
	>61	42.9	28.6	28.6
Education	No school	62.5	12.5	25.0
	Primary school	58.8	23.5	66.7
	Junior school	50.0	16.7	33.3
	High school	50.0	14.3	35.7
	Tertiary	.0	33.3	17.6
Farm experience	<5years	40.0	20.0	40.0
	6- 10	33.3	33.3	33.3
	11- 15	33.3	33.3	33.3
	16- 20	68.2	9.1	22.7
	>21	50.0	20.0	30.0
Who is involved in farming	Myself	63.9	33.1	40.3
	Relation/employee	36.2	8.4	17.6

In Ga-kibi village 68.2% of the respondents interviewed had 16 to 20 years' beef cattle rearing experience; while in Norma and Mogalakwena the situation is different with 9.1% and 22.7% respectively. Nevertheless, 40% in Ga-kibi, 20% in Norma and 40% in Mogalakwena had less than 5 years of beef cattle rearing experience. On the whole, majority of the respondents undertake farming activities as a means of livelihood although a small number of them are pensioners who derive extra income from monthly pension. However, there is a substantial variation in the variable: who is actually involved in beef cattle farming? The result revealed that 63.9% of respondents in Ga-kibi, 33.1% and 40.3% in both Norma and Mogalakwena were actively involved in farming respectively (Table 1). Overall, the respondents in Ga-kibi, Norma and Mogalakwena seldom use hired labour with exception of few farmers that are either too old or physically challenged. The result shows that 36.2% of the respondents in Ga-kibi, 8.4% in Norma and 17.6% in Mogalakwena implore and use the services of relations or hired labour.

3.2 Farmers Perception of Production and Marketing Constraints

The variables used to assess beef farmers' perception of production and marketing constraints were: pest and diseases, water stress, market infrastructure, market access, price fixing, labour and livestock theft.

3.2.1 Pest and Diseases

The prevalence of pest and diseases constitute a major constraint to cattle production exacerbated by poor production practices. Almost all farmers in Ga-kibi, Norma and Mogalakwena seldom apply initial control measures but usually adopt treatment procedures when cattle are infected. Overall, 50% of beef cooperative farmers in Ga-kibi, 20% in Norma and 50% in Mogalakwena asserted that pest and diseases limits the production of livestock in the area (Figure 1). According to the respondents, high mortality of their beef cattle is induced by the incidence of tick resulting in the occurrence of Heart water disease.

Table 2. Logistics regression showing years of farm experience and perception of pest and diseases as constraints

Variables in the Equation:							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Fexp			2.526	2	.283	
	Fexp(1)	1.317	.895	2.166	1	.141	3.733
	Fexp(2)	.480	.734	.427	1	.514	1.615
	Constant	-.847	.690	1.508	1	.220	.429

Variable(s) entered on step 1: Fexp=Years of farm experience.

The results showed that farmers with farm experience of more than 20 years are 2.62 (1/.381) times more likely to perceive pest and diseases as a constraint as compared to farmers with less farm experience (Table 2). Also, farmers with farm experience ranging from 11-20 yrs are 0.94 times more likely to perceive pest and diseases as a constraint than farmers with less than 11 years farm experience. Therefore, farmers' assessment of constraint increases with number of years of farming experience.

3.2.2 Water Stress

The availability of adequate water throughout the year for livestock in the Ga-kibi, Norma and Mogalakwena has been a serious concern for the beef cooperative farmers. Most farmers rely on harvested rain water to cushion water shortages. The sources of water for livestock are bore hole (well), streams or rivers which are in most cases far from the grazing camps. Few of the available boreholes located in the grazing camps are either manually or electrically operated. In Ga-kibi, 50% of the beef cooperative farmers agreed that water stress constitute a serious constraint in their livestock enterprise while 28% and 50% of the respondents in Norma and Mogalakwena respectively also concurred that the problem of water remains unabated in the area. The results of the logistics regression (Table 3) revealed that farmers with farm experience of more than 20 years are 1.78 (1/.563) times more likely to perceive water stress as a constraint than farmers with farm experience of less than 10 years. The findings revealed that the ability of farmers to perceive water stress as a constraint increases with number of years of farming experience.

Table 3. Logistics regression showing years of farm experience and perception of water stress as a constraint

Variables in the Equation							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	fexp			.600	2	.741	
	fexp(1)	-.074	.846	.008	1	.930	.929
	fexp(2)	-.575	.993	.336	1	.562	.563
	Constant	1.386	.791	3.075	1	.080	4.000

a. Variable(s) entered on step 1: fexp=Years of farm experience.

3.2.3 Market Infrastructures

The Comprehensive Agricultural Support Programme (CASP) has been mandated to build infrastructures like erection of fences, auction sales pens and ramps for loading and off-loading livestock (NDA, 1998). In the study areas, it is fascinating to note from the focus group discussion that the beef cooperative farmers improvised some infrastructures using indigenous knowledge. In Norma area for example, a group of farmers excavated and manually operated wells (local bore hole) to serve as source of water for their livestock. Marketing infrastructures like feedlots, abattoirs, auction sales pens and ramps are unavailable in the area. Result reveals that in Ga-kibi 68%, Norma 11% and Mogalakwena 21% of the respondents agreed that market infrastructure is a major barrier in beef cattle sales. In Ga-Kibi, the beef cooperative farmers asserted that the unsettled impasse between the Local Authority and the Limpopo Department of Agriculture (LDA) has negatively affected the successful completion and commissioning of the feedlot in the area. In Mogalakwena for instance, the situation is different; there are three pumping machines donated by the LDA, but these machines are not utilized because of perennial water problem. The source of water available for livestock in the area is either dried up or inadequate in volume to allow submersible pumping machine to function. Conversely, the beef cooperative farmers in Norma posited that in 2010 the Department of agriculture (LDA) installed submersible water pumps for the existing bore hole in Norma area but it became defective after six months of operation.

Table 4. Logistics regression showing years of farm experience and perception of market infrastructure ras a constraint

Variables in the Equation							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Fexp			1.246	2	.536	
	Fexp(1)	-.014	.740	.000	1	.984	.986
	Fexp(2)	-.693	.886	.611	1	.434	.500
	Constant	.847	.690	1.508	1	.220	2.333

a. Variable(s) entered on step 1: Fexp= Years of farm experience.

The perception of market infrastructures as a constraints by beef cooperative farmers was investigated (Table 4) and the result revealed that beef cooperative farmers with longer years of experience (20 years or more) are 2.00 (1/.500) times more likely to perceive market infrastructures as a constraint than farmers with less farm experience.

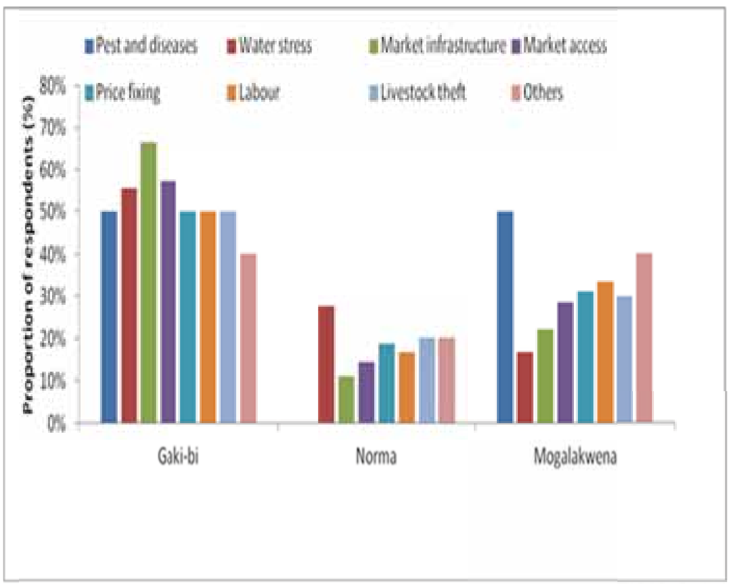

Figure 1. Farmer’s perception of production and marketing constraints

3.2.4 Market Access

In South Africa, there are presently five approved networks for sales and marketing of livestock. The approved networks are abattoirs, market agents (speculators), feedlots, butcheries and private sales (NDA, 1998). The beef cattle industry has grown from a controlled environment to one that is completely deregulated today. Numerous policies, such as differences between organised and unorganised areas; payment of levies by producers; regulations on the building of abattoirs; auctioning of animals according to grade and weight was a common feature in the beef cattle industry prior to deregulation of agricultural market. Subsequently, with the deregulation of the agricultural marketing, the prices of beef cattle are settled by the forces of demand and supply. The inclusion of the smallholder farmers in the value chain management is essential for the survival of rural agricultural production and marketing (Van Tilburg, 2012). Since the deregulation of the agricultural market, smallholder farmers have persistently been marginalised in the value chain. Liberalization and deregulation of agricultural markets posed restraints that debar smallholder farmers' market access for agricultural products. In this study however, there was no significant difference in the perception of market access as a constraint in the three villages. In Ga-kibi 56% (Figure 1) of the respondent were of the view that access to market has been a major hindrance in the sales of their culled beef cattle. In Norma 17% of the respondents agreed that access to market remains their concern while in Mogalakwena, 27% (Figure 1) also asserted that inadequate access to the market is an obstacle to the sale of beef cattle despite the existence of cooperatives. According to Makhura and Mokoena (2003) institutional provision along the value chain seldom prioritize the needs and aspirations of the smallholder farmers who are already marginalised and thus increased the barriers to accessing markets. The beef cooperative farmers' access to market in the area was identified as a major problem. However, farmers with farm experience of 20 years and above were 4.00 (1/.250) times more likely to consider market access as a constraint than farmers with less farm experience (Table 5).

Table 5. Logistics regression showing years of farm experience and perception of market access as a constraint

Variables in the Equation:							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Fexp			2.509	2	.285	
	fexp(1)	-.351	1.113	.100	1	.752	.704
	fexp(2)	-1.386	1.213	1.305	1	.253	.250
	Constant	2.197	1.054	4.345	1	.037	9.000

a. Variable(s) entered on step 1: fexp= Years of farm experience.

3.2.5 Price Fixing

Considering the parameters used for livestock quality and price estimation, beef cooperative farmers are seldom unable to attract reasonable prices for their beef animals in the formal market. Beef cattle are graded as class 'C' if it has reached the matured age which implies that such beef cattle will fetch the farmer the lowest price per kilogramme. Too fat or lean beef cattle are not also desirable by potential buyers or speculators. Poor management practices coupled with ill-health also accounts for low prices of beef animals (NDA, 2008). Overall, 50% of beef cooperative farmers' in Gaki-bi asserted that price fixing by the speculators was a major problem militating against beef production (Figure 1). In Norma, 20% were of the view that price fixing by speculators poses a major problem while 30% of respondents in Mogalakwena had similar perception. However, majority of the respondents in Ga-kibi, Norma and Mogalakwena asserted that the concept of grading by speculators before price fixing is not very clear. The grading of beef cattle by speculators for ease of determining the price has also placed farmers in a disadvantaged position. The result revealed that in the three areas, farmers criticized the grading methods used in determining the prices of their beef cattle. This lack of transparency in estimating prices often leads to suspicion and most beef cooperative farmers consider this phenomenon as exploitative.

Table 6. Logistics regression showing years of farm experience and perception of price fixing as a constraint

Variables in the Equation							
		B	S.E.	Wald	Df	Sig.	Exp(B)
Step 1[a]	Fexp			.251	2	.882	
	fexp(1)	-.085	.739	.013	1	.908	.918
	fexp(2)	-.377	.895	.178	1	.673	.686
	Constant	.847	.690	1.508	1	.220	2.333

a. Variable(s) entered on step 1: fexp= Years of farm experience.

The results showed that farmers with more than 20years farm experience are 1.46 (1/.686) times more likely to perceive price fixing as a constraint than farmers with less than 10yrs farm experience (Table 6).

3.2.6 Labour

Labour is readily available in Ga-kibi, Norma and Mogalakwena villages. Results revealed that 50% of the respondents in Ga-kibi use direct labour involving families and relations. In Norma, 19% of the respondents agreed that labour was not a major constraint while in Mogalakwena 31% (Figure 1) also agreed that labour does pose a constraint. Overall, beef cooperative member involves members in sharing their normal routine duties. For instance adhoc committees or groups are form to attend to the mending of broken fences and directing livestock to the grazing camps.

Table 7. Logistics regression showing years of farm experience and perception of price fixing as a constraint

Variables in the Equation:							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	Fexp			3.288	2	.193	
	fexp(1)	-1.099	1.307	.707	1	.401	.333
	fexp(2)	.693	.833	.693	1	.405	2.000
	Constant	-1.386	.791	3.075	1	.080	.250

a. Variable(s) entered on step 1: fexp=Years of farm experience.

However, results showed that farmers with higher farm experience (≥20years) are 0.5 (1/2.00) times less likely to perceive labour as a constraint than farmers with than 10years farm experience (Table 5).

3.2.7 Livestock Theft

Livestock pilfering was identified as one of the constraints to beef cattle production in the study areas. Stock pilfering causes economic loss, misfortune and hardship to the farmers. It is also a social malaise as this may reduce the level of trust among community members. Initiatives like branding and ear tagging of livestock to ameliorate pilfering failed because speculators and individuals are allowed to purchase livestock at farm gate. In Ga-kibi, 50% (Figure 1) of the respondents asserted that pilfering of their stock constitute a setback in their farming business while in Norma, (20%) and Mogalakwena (30%) of the respondents interviewed agreed that stock pilfering poses a problem in their production and marketing. As an interim measure, farmers in the study areas agreed to the introduction of 'farmers watch' and the formation of 'security committee' to reduce the incidence of stock pilfering.

Livestock theft:

Table 6. Logistics regression showing years of farm experience and perception of price fixing as a constraint

Variables in the Equation:							
		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1[a]	fexp			2.940	2	.230	
	fexp(1)	2.043	1.192	2.938	1	.087	7.714
	fexp(2)	1.572	1.085	2.097	1	.148	4.814
	Constant	-2.197	1.054	4.345	1	.037	.111

a. Variable(s) entered on step 1: fexp= Years of farm experience.

The pilfering perception were investigated and results revealed that farmers with higher farm experience do not perceive pilfering or theft as a constraints as much as farmers with less farm experience. The results showed that farmers with more farm experience (20 years) are 0.21 (1/4.814) times more likely to perceive livestock theft as a constraint. While farmers with farm experience of 11-20 years were 0.13 times more likely to perceive livestock theft as a constraint.

4. Farmers, Perception of Beef Cattle Production as an Income Generating Source

In South Africa, livestock account for 45% of the national agricultural domestic product. In Limpopo province, beef cattle constitute 17.6% of farming operation (Stats SA, 2002). Beef cattle have a lot of potential for development and income generation in the study area. The variables used in determining farmer's perception of beef cattle as income generating source were: main source of income, auxiliary source of income and minor source of income. In Ga-kibi, 55% (Figure 2) of the respondents interviewed asserted that beef cattle production was their main source of livelihood. In Norma, 19% of the respondents depend on beef cattle production as an income generating source while in Mogalakwena, 26% depends on beef cattle as their main source of income. However, off-farm activities in the three study areas were not too pronounced as some of the farmers are either retiree or are involved in small stock production like goats and sheep. Livestock farming therefore, is a major source of income in the area.

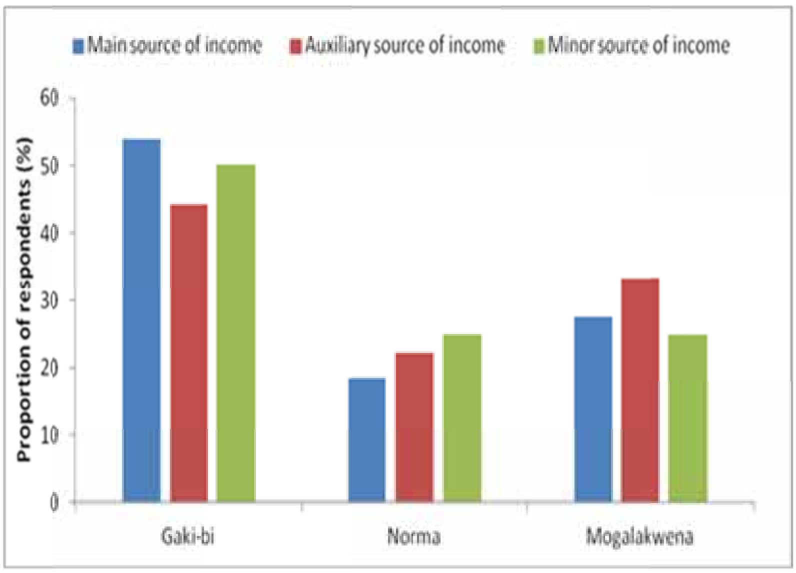

Figure 2. Farmers, perception of beef cattle production as an income generating source

5. Speculators and Marketing in Ga-kibi, Norma and Mogalakwena

Although other variables were identified as constraints, price fixing by speculators who are 'branded traders' were tagged by majority of respondents as a key limiting factor in beef cattle production and marketing in the three areas (Figure 2). Market speculators have access to infrastructures that allow them to purchase beef cattle from farmers at low prices and resale usually at a higher price. The four speculators interviewed asserted that prices are determined by the forces of demand and supply with abattoirs and feedlots staff monitoring the pace.

These prices that are considered ideal are accepted as the prevailing price for the week. However, the approach to the price fixing is not usually clear to the beef cooperative farmers hence they consider the speculator as falsehearted. South Africa Agricultural Research for Development (SA ARD, 2006) noted that this form of marketing outlet is dominant in many rural areas of BLM and it is considered as one-sided method of steering business. However, as unsuitable as this marketing channel may be, it has its own benefits, for instance farmers do not incur marketing costs (cost of sales) because sale tariff or commission is not charged as sale take place at farm gate or their homestead without cost of transporting the beef cattle.

Nevertheless, the assertion of the speculators was that they also incur some feeding cost in the feedlots and abattoir before slaughter. Costs are predetermined before prices are fixed and usually the beef cooperatives farmers lack idea on value adding techniques which entails feeding the beef cattle to improve the live weight, carcass weight, dressing percentage and meat quality which are potential aspects that are of interest to abattoirs, slaughter houses and consumers. It is important that the speculators continue to feed these beef cattle as off-feed before slaughter can affect their live weight. For instance, studies have exemplified that livestock generally loses approximately 1% to 3% of their live weight when kept off-feed for 24 hours prior to slaughter (Bider et al., 1997). The abattoirs usually do not finish the slaughtering of all purchased beef cattle in a day and therefore; continue feeding until it is slaughtered. Depriving beef cattle of feed prior to slaughter is likely to reduce carcass weight, and therefore, results in low returns especially when they are sold on dead-weight basis; and the magnitude of this lose increases with time off-feed. However, speculators sometimes take advantage of the peculiar social-economic misfortune of the farmers to obtain lower prices far below the market value of the beef cattle. For instance, the speculators agreed that prices of beef cattle are cheaper when most farmers are returning their children back to school. Farmers are constrained to sell below the marketing price and often they swop their small stock (goats and sheep) for farm implements like wheel barrows which are used for fetching water for their livestock and household. Farmers that need funds for funeral or other unforeseen contingencies may also sell their beef cattle below the prevailing market prices. Distress sales may also result from insufficient fodder to feed the beef cattle. Another teething problem is that most speculators are not ready to go to remote areas because of poor road network associated with rural areas and high crime rate since market transactions are done with cash. Nevertheless, the speculators remain to be hunted after to purchase these beef animals from the farmers.

5.1 Improving the Performance of Beef Cooperatives in the Study Area

Enhancing the functioning of beef cooperatives requires farmers training and mentorship. Beef cooperatives farmers were not amply informed of the grading modalities and subsequent price fixing for livestock; it is therefore, imperative to include the farmers in the participatory process. The quest for auctioning are desirable but the modalities should be made clear to the livestock farmers. The addition of the smallholder farmers in the value chain administration is essential for the survival of rural agricultural cooperatives and marketing. Beef cooperatives farmers must form strategic partnership with the National Emergent Red Meat Producers' Organisation (NERPO) whose mandate amongst other things aims to develop the agricultural sector and ensure the right involvement of smallholder farmers within the ambit of agribusiness sector for long term sustainability. The beef cooperatives farmers must be encouraged to liaise with the Comprehensive Agricultural Support Programme (CASP) which has hitherto been assigned the responsibilities of infrastructural development for smallholder farmers in South Africa. Government must be consistent in offering technical assistance in areas like pest and diseases control, water resources management, market infrastructures and access, grading and stock pilfering. Service delivery must be fast tracked to indigent farmers.

6. Conclusion

The agitation for more investment in smallholder agriculture and farmers cooperatives has been gathering momentum and support and has been recognised as an avenue for poverty alleviation. However, there has been debate about its effectiveness since most smallholder farmers have no adequate access to the market as exemplified in this study. The results of the study reveal that beef cooperative farmers in Ga-kibi, Norma and Mogalakwena are saddled with the problem of pest and disease, water stress, market infrastructures, inadequate market access, unfair practices of price determination by speculators and stock pilfering. Beef cattle enterprise has a lot of prospect for development and income generation in the study area. Majority of the respondents depends on the beef cattle farming as an income source and as a means of livelihood. However, the daunting task of farmers embracing the principles and practice of cooperatives still persist in the study area. The study exemplified inadequate understanding of the purpose of cooperative, their obligations and rights, and how to manage the cooperative business in the area. Cooperatives' inability to provide transport services, inadequate commitment and lack of understanding of members' roles were causative factors. The solution to poverty reduction requires involving a greater part of the rural poor in agricultural cooperative activities that will enhance sufficient income. However, several factors have hindered the performance of smallholder cooperatives in developing countries.

Acknowledgement

The author gratefully acknowledge the International Centre for Development Oriented Research in Agriculture (ICRA), Limpopo Department of Agriculture (LDA), Blouberg Local Municipality (BLM), University of Fort Hare, University of Limpopo, University of Venda and Agricultural Research Council (ARC) for funding the fieldwork that culminated in the data collected for this paper. I also sincerely acknowledge the three tribal communities Ga-kibi, Norma and Mogalakwena and the Agricultural Research for Development (ARD) team 2012, of which the author is a member for their invaluable contributions.

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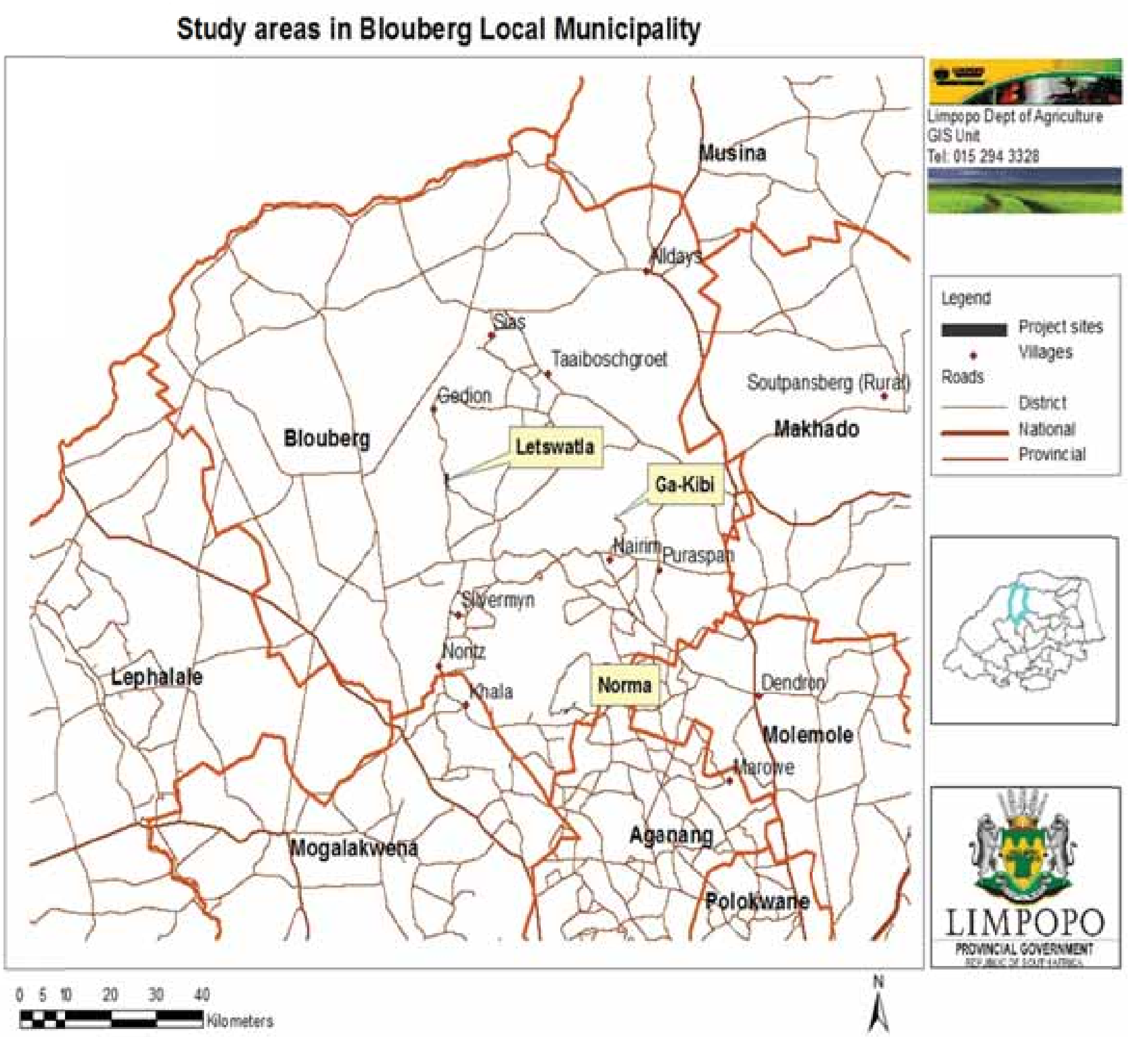
Appendix 1: Map of the study area



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Effect of Nitrogen Source and Weed Management Systems on No-Till Corn Yields

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Abstract

Field research was conducted at upstate Missouri to evaluate the impact of weed management systems and pre-plant nitrogen source selection [polymer-coated urea, (PCU); anhydrous ammonia (AA), urea, and ammonium nitrate (AN)] and side dressed urea ammonium nitrate (UAN) at 168 kg N ha^{-1} on no-till corn grain yield and weed growth. Small-seeded broadleaf weed heights responded differently to PCU and anhydrous ammonia in the two years of study. Corn heights were greater with AN and urea compared to PCU, AA, and side dressed UAN 7 to 9 weeks after planting. Nitrogen fertilizer source selection and weed management system affected total weed biomass (giant foxtail, common waterhemp, and common lambsquarters) at physiological maturity of corn. However, these factors showed no interactive effect on corn grain yields. An early postemergence application of atrazine + dimethenamid-*P* + glyphosate reduced total weed biomass 86% and 92% compared to atrazine + dimethenamid-*P* applied preemergence following AA and the non-fertilized control, respectively. A two-pass postemergence system (glyphosate followed by glyphosate) had 74 to 79% greater weed biomass compared to residual systems when following PCU. All weed management systems increased yield 1.5 to 5.09 Mg ha^{-1} compared to the non-treated control, and no yield difference was observed among weed management systems. PCU, AA, and side dressed UAN are preferred over broadcast urea for integrated weed management of no-till corn production in this region.

Keywords: ammonium nitrate, anhydrous ammonia, competition, polymer coated urea, urea, urea ammonium nitrate, weed removal

1. Introduction

Early in the growing season, weeds can accumulate N rapidly, which can contribute to early-season interference and subsequent yield loss in corn (*Zea mays* L.) (Teyker et al., 1991; Davis & Liebman, 2001; Evans et al., 2003a, 2013b; Cathcart & Swanton, 2004; Harbur & Owen, 2004; Lindquist et al., 2010), wheat (*Triticum aestivum* L.) (Blackshaw et al., 2002; Blackshaw et al., 2004), rice (*Oryza sativa* L.) (Ampong-Nyarko & De Datta, 1993a, 1993b), and canola (*Brassica napus* L.) (Blackshaw et al., 2011). Weeds can reduce soil NO_3-N up to 50% in corn (Lindquist et al., 2010). Several integrated weed management studies (Walker & Buchanan, 1982; Di Tomaso, 1995) have investigated nitrogen because direct uptake by weed species may affect control (Kim et al., 2006) and grain yields depending on fertilizer rate (Evans et al., 2003a, 2003b; Cathcart & Swanton, 2004; Lindquist et al., 2010), placement (Blackshaw et al., 2002; Blackshaw et al., 2004), timing (Blackshaw et al., 2004; Harbur & Owen, 2004), and source (Teyker et al., 1991; Davis & Liebman, 2001; Blackshaw et al., 2011). In corn, nitrogen fertilizer recommendations and the impact on weed interference may depend on the weed species (Harbur & Owen, 2004). Weed management has been more critical as rates of N were reduced (Evans et al., 2003b; Cathcart & Swanton, 2004). However, with the introduction of enhanced efficiency fertilizers such as polymer-coated urea (PCU), N source selection (Teyker et al., 1991) may be an important component of integrated weed management.

Best management practices are available to help farmers make informed decisions on increasing N use efficiency in corn (Scharf & Lory, 2006). A preplant surface broadcast application of urea in the absence of a urease or nitrification inhibitor typically is not recommended for no-till corn production due to risk of loss (Ferguson &

Kissel, 1986; Rochette et al., 2009) and yield loss (0.42 to 0.81 Mg ha^{-1}) compared to ammonium nitrate (AN) (Stecker et al., 1993). However, recent regulations have decreased the availability of AN to farmers, a situation that has prompted industry to develop technology that increases efficiency of urea fertilizers. Polymer-coated urea is a controlled-release urea fertilizer that allows farmers to broadcast apply preplant N and reduce gaseous fertilizer loss such as N_2O up to 49% in no-till compared to non-coated urea (Rochette et al., 2009; Halvorson et al., 2010). In canola, a preplant application of controlled-release N fertilizers and/or deep placement of N reduced overall weed growth and N uptake in the biomass of weeds, which could mitigate crop-weed competition for NO_3-N in soil (Blackshaw et al., 2011). However, minimizing weed growth by limiting soil N availability may reduce the effectiveness of herbicide applications (Evans et al., 2003b) due to reduced interception and retention of herbicides on weeds (Kim et al., 2006). Changes in N management might also affect the critical period for weed control (Evans et al., 2003a). Therefore, N management practices in no-till corn that promote controlled-release of available N early in a growing season may require more intensive weed management systems. Such systems could be similar to those used in research evaluating low N rates (Evans et al., 2003a, 2003b; Cathcart & Swanton, 2004; Lindquist et al., 2010) to obtain the potential yield benefits derived from reduced weed-crop competition and the subsequent increase in available soil N for crop uptake.

The Midwestern U.S. contains more than 4 million ha of claypan soils (Anderson et al., 1990). Low hydraulic conductivity in the claypan subsoil layer minimizes the potential for N loss through leaching, but it increases the potential for denitrification loss due to its high propensity for extended periods of soil saturation. Also, the potential can be large for volatilization loss from surface-applied urea-based fertilizers directly after application due to warm, moist soil conditions in the spring (Ferguson & Kissel, 1986). Maximizing no-till corn yields in a claypan soil requires N fertilizer with the lowest potential for denitrification and volatilization loss (Nash et al., 2012a). Injecting anhydrous ammonia (AA) at depth reduces the potential for volatilization loss and generally presents the lowest risk of yield-limiting denitrification loss compared to other conventional N fertilizers (Scharf & Lory, 2006; Nash et al., 2012a). Addition of a polymer coating around urea prills with PCU results in a slow release of available N over time. This reduces volatilization and denitrification loss compared to conventional urea fertilizers (Rochette et al., 2009; Halvorson et al., 2010), and increases grain yields in high-risk areas of fields compared to non-coated urea (Noellsch et al., 2009; Motavalli et al., 2012). Reduced gaseous N loss with AA and PCU might result in greater N availability throughout the growing season and increase overall weed growth by the time corn reaches physiological maturity.

Weed control in no-till corn with weed management systems is expected to vary, depending on the N source. This is due to aggressive weed growth that might result from readily available N sources such as non-coated urea or AN. The availability of N to the crop or weed in a no-till production system might depend on the N source selection because some sources are placed below the soil surface (AA) or are controlled-release (PCU) (Blackshaw et al., 2004; Blackshaw et al., 2011). Polymer-coated urea is a controlled-release N source that might limit early weed growth due to this technology's slow N release properties. Similarly, AA is banded 15 to 20 cm below the soil surface, and root growth is necessary to access this N source. In other research, AA and PCU yields were similar in high-risk N loss areas of a field (Noellsch et al., 2009; Motavalli et al., 2012). It may be imperative to have better early season weed control with an N source that is placed below the soil surface in a no-till production system.

Research conducted on how N sources and weed management systems affect crop production is very scanty. The handful of studies includes primarily an N source study on sweet corn (Davis & Liebman, 2001), greenhouse experiment with corn (Teyker et al., 1991), and no-till canola (Blackshaw et al., 2011). Most field corn research looks at conventional tillage systems (Davis & Liebman, 2001; Evans et al., 2003a, 2003b; Cathcart & Swanton, 2004; Lindquist et al., 2010). However, no research has evaluated how AA or new controlled-release PCU fertilizer affects no-till corn production with common weed management systems. We hypothesized that broadcast preemergence-applied PCU, deep placement of AA, and side dressed urea ammonium nitrate (UAN) would have shorter weeds than faster release N sources such as AN and non-coated urea. This would subsequently affect no-till corn grain yields, depending on which weed management systems were implemented. This research sought to determine how weed management systems and preplant N source selection affects no-till corn grain yield, weed heights, and weed control.

2. Materials and Methods

A field trial with three replications in 2006 and four replications in 2007 was conducted using 3.1 by 10.7 m plots at the University of Missouri Greenley Research Center near Novelty (40º01′ N, 92º11′ W). The study employed a split-plot design, with N source as the main plot and weed management system as the sub-plot. All N sources [AA, urea, PCU (ESN, Agrium, Inc.), AN] were applied preplant at 168 kg N ha^{-1}. Urea ammonium nitrate was applied in a dribble-band at 168 kg N ha^{-1} as a side dress best management practice control treatment (Scharf & Lory, 2006), and a non-fertilized control was included. The soil was a Putnam silt loam (fine, smectitic, mesic, Vertic Albaqualfs). No maintenance fertilizer was applied because soil test data showed that nutrient concentrations were high to very high (Table 1) (Buchholz, 2004).

Table 1. Soil test values and corn management practices

Management practice†	2006	2007
Soil test values		
Soil organic matter (g kg^{-1})	33‡	25
Cation exchange capacity (meq/100 g)	15.4	13.5
pH (0.01 *M* $CaCl_2$)	7.0	6.1
Bray I P (kg ha^{-1})	118	49
Exchangeable (1 *M* NH_4AOc)		
K (kg ha^{-1})	507	284
Ca (kg ha^{-1})	5990	4740
Mg (kg ha^{-1})	404	430
Planting date	28 Apr.	23 Apr.
Hybrid	DKC60-19	DKC60-19
Seeding rate (seeds ha^{-1})	74,100	76,100
Tillage	No-till	No-till
Fertilizer date		
Preplant N	28 Apr.	23 Apr.
Side dress UAN (30-cm tall corn)	5 June	29 May
Herbicide treatments (rate)‡		
§Atrazine (2.2 kg ai ha^{-1}) + glyphosate (0.84 kg ae ha^{-1})		
POST (10 cm tall weeds)	13 June	25 May
Atrazine + *S*-metolachlor + mesotrione premix (2.2 + 0.8 + 0.2 kg ai ha^{-1}) + NIS (0.25% v/v)		
POST (5 to 10 cm tall weeds)	9 June	17 May
Atrazine + dimethenamid-*P* premix (2.1 + 1.1 kg ai ha^{-1}) + NIS (0.25% v/v)		
POST (3 to 5 cm tall weeds)	2 June	10 May
Glyphosate (0.84 kg ae ha^{-1}) fb glyphosate (0.84 kg ae ha^{-1})		
POST (10 cm tall weeds) fb	13 June fb	25 May fb
POST (10 cm tall weeds)	1 July	24 June
Atrazine (2.2 kg ai ha^{-1}) fb glyphosate (0.84 kg ae ha^{-1})		
PRE fb POST (10 cm tall weeds)	3 May fb 13 June	24 Apr. fb 25 May
Atrazine + dimethenamid-*P* premix (2.1 + 1.1 kg ai ha^{-1}) + glyphosate (0.84 kg ae ha^{-1})		
POST (10 cm tall weeds)	13 June	25 May

†Abbreviations: fb, followed by; NIS, non-ionic surfactant (Activator-90, a mixture of alkylpolyoxyethylene ethers and free fatty acids, Loveland Industries Inc., Greeley, CO); POST, postemergence; PRE, preemergence; and UAN, urea ammonium nitrate.

‡All plots received a burndown application of glyphosate at 0.84 kg ae ha^{-1} on 20 Apr. 2006 and 23 Apr. 2007. Weed populations averaged 24 common waterhemp m^{-2}, 30 common lambsquarters m^{-2}, and 5 giant foxtail m^{-2} in the non-treated control.

§Herbicide chemical names: atrazine, 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine; dimethenamid-*P*, 2-chloro-N-[(1-methyl-2-methoxy)ethyl]-N-(2,4-dimethyl-thien-3-yl)-acetamide); glyphosate, *N*-(phosphonomethyl)glycine formulated as Roundup WeatherMAX®; mesotrione, 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione); and *S*-metolachlor, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide.

Weed management systems included a non-treated weedy check, weed-free control, as well as standard preemergence only, early postemergence, and two-pass weed management systems. Herbicide application treatments, timings, and rates are reported in Table 1. All herbicide treatments were applied with a CO_2-propelled backpack sprayer calibrated to deliver 140 L ha^{-1}. The spray boom was equipped with 8002 flat-fan nozzles (Spray Systems Co., North Avenue, Wheaton, IL) spaced 38 cm apart and positioned 41 cm above the canopy. In the non-treated control, weed populations averaged over 2006 and 2007 were 24 common waterhemp [*Amaranthus tuberculatus* (Moq.) J.D.Sauer] m^{-2}, 30 common lambsquarters (*Chenopodium album* L.) m^{-2}, and 5 giant foxtail (*Setaria faberi* Herrm.) m^{-2}. In the non-treated control, three plants of each broadleaf weed species (common waterhemp and common lambsquarters) were marked with plastic stakes prior to spraying herbicides and heights were measured weekly starting 4 weeks after planting (WAP) in 2006 and 3 WAP in 2007 until 6 to 7 WAP. Corn heights in the weed-free control were measured 7 to 9 WAP to evaluate corn growth differences when plants were under high demand for N (Scharf & Lory, 2006). Season-long weed control was evaluated by harvesting two, 30- by 76-cm quadrants from each plot near physiological maturity in early September (Ritchie et al., 1993). Weeds were separated by species, dried, and weighed. The primary late-season weeds were giant foxtail, common waterhemp, and common lambsquarters.

A small-plot combine (Massey Ferguson 10, Kincaid Equipment Manufacturing, Haven, KS) harvested and weighed each plot's centermost two rows. Seed moisture was determined at harvest and adjusted to 150 g kg^{-1} before data analysis. All data were subjected to ANOVA using PROC GLM (SAS, 2013, vers. 9.3) and combined over site-years in the absence of interactions. Means were separated using Fisher's Protected LSD at P = 0.01. Standard errors of the means were presented for weed- and corn-height measurements.

3. Results and Discussion

3.1 Precipitation

Overall precipitation during the 2006 and 2007 growing seasons (550 mm) was similar (Figure 1). In 2006, drier spring conditions were favorable for optimal stand establishment. However, poorly drained claypan soils remained extremely wet during the spring of 2007. These conditions are conducive to gaseous nitrogen loss of urea in no-till corn on claypan soils (Nash et al., 2012a). The average temperature was 20.2ºC in 2006, and 21.2ºC in 2007 (Sandler et al., 2012). Throughout Sep. and Oct. 2006, temperatures were abnormally low, averaging 11.2ºC.

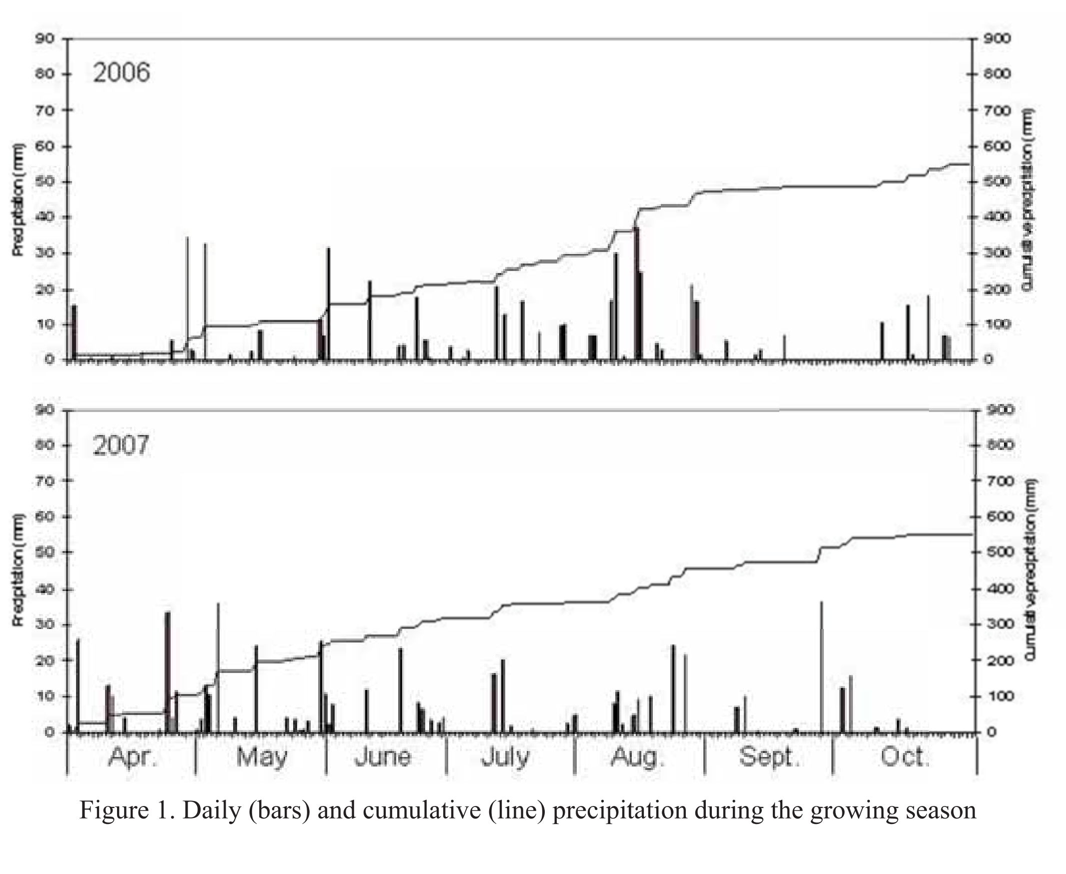

Figure 1. Daily (bars) and cumulative (line) precipitation during the growing season

3.2 Weed Heights

In 2006, common waterhemp heights with urea (Figure 2) and side dressed UAN (data not presented) were similar to the non-fertilized control. Plant heights were 3 to 8 cm greater 6 to 7 WAP when PCU, AA, or AN were applied, compared to the non-fertilized control (Figure 2A). Common waterhemp was tallest when PCU was applied compared to other N sources and the non-fertilized control 7 WAP. However, in 2007, soluble N sources such as AN and urea had the tallest common waterhemp plants 5 to 6 WAP followed by PCU (Figure 2B). Anhydrous ammonia and side dressed UAN (data not presented) were similar to the non-fertilized control. Common waterhemp height was affected by N source 3 to 7 WAP; however, in an Iowa study, common waterhemp height was not affected by AN application timings (Harbur & Owen, 2004).

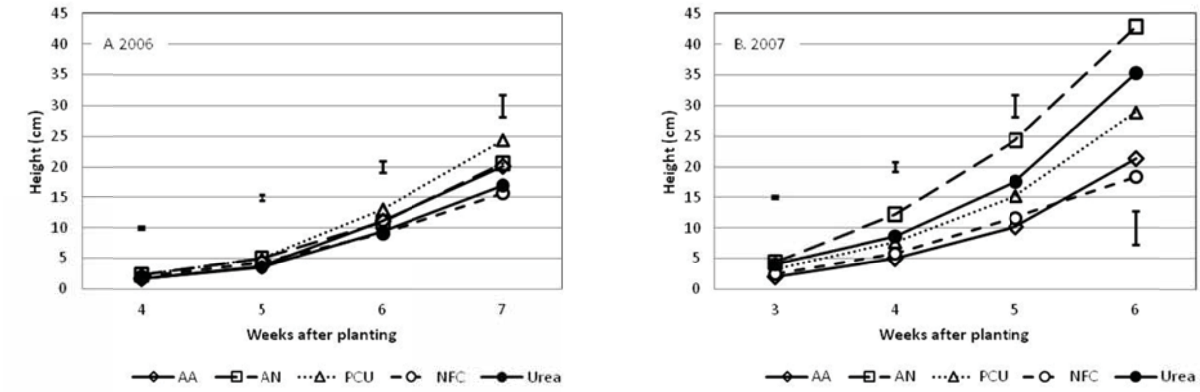

Figure 2. Common waterhemp height response to preplant nitrogen sources (AA, anhydrous ammonia; AN, ammonium nitrate; NFC, non-fertilized control; PCU, polymer-coated urea; and urea) at 168 kg N ha^{-1} in the non-treated weed management control 4 to 7 weeks after planting (WAP) in 2006 (A) and 3 to 6 WAP in 2007 (B). Vertical bars represent the standard error

Common lambsquarters heights with side dressed UAN (data not presented), preplant urea, and AA (Figure 3A) were similar to the non-fertilized control in 2006. Heights were similar among N sources 6 WAP, but were over 5 cm taller with PCU compared to other N sources and the non-fertilized control. In 2007, there was no difference in common lambsquarters heights between PCU, urea, and AN fertilizer sources. However, heights were similar to the non-fertilized control when AA was applied preplant (Figure 3B) or UAN was side dressed (data not presented).

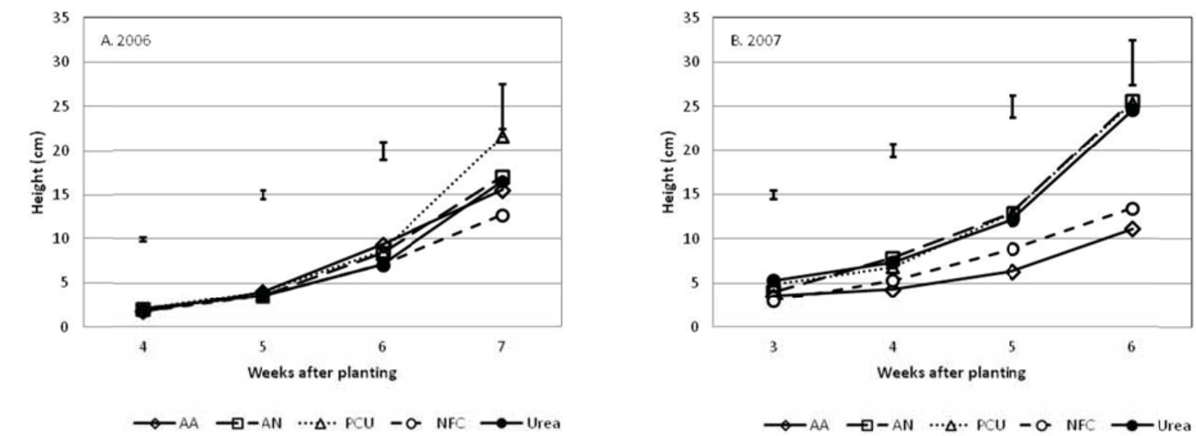

Figure 3. Common lambsquarters height response to preplant nitrogen sources (AA, anhydrous ammonia; AN, ammonium nitrate; NFC, non-fertilized control; PCU, polymer-coated urea; and urea) at 168 kg N ha^{-1} in the non-treated weed management control 4 to 7 weeks after planting (WAP) in 2006 (A) and 3 to 6 WAP in 2007 (B). Vertical bars represent the standard error

These data indicate that various N sources affected small-seeded broadleaf weed growth differently, depending on placement (AA injected below the soil surface or UAN dribble banded between the row) as well as PCU's slow-release properties. These results are similar to a four year, spring-wheat study in which subsurface banded or point-injected liquid N in the spring generally resulted in lower weed biomass compared to a surface broadcast application (Blackshaw et al., 2004). Herbicide label recommendations are based on weed heights, and N source selection might slightly affect the timing requirement for a postemergence herbicide based on the weed's growth. This has been shown in other research evaluating N rates (Evans et al., 2003a). In this experiment, herbicide treatments were applied at the same time (Table 1) to avoid confounding environmental conditions at the time of application.

3.3 Corn Heights

No significant differences occurred between N sources and year for corn heights 7 to 9 WAP for the weed-free control; therefore, data were pooled over years. Corn height in the weed-free control was similar for the non-fertilized control, AA (Figure 4), and side dressed UAN (data not presented). This was probably because the deep placement of AA and the between-row side dress application of UAN slowed overall corn growth slightly compared to the other N sources during a period of rapid N uptake (Scharf & Lory, 2006). This could affect canopy development and the crop's ability to interfere with weed growth and germination of late-emerging weeds. Higher N rates have helped corn plants compete with weeds more effectively (Evans et al., 2003b; Cathcart & Swanton, 2004). Corn was slightly taller (8 to 12 cm) with AN and urea compared to PCU 7 to 9 WAP. This was probably due to PCU's controlled-release of urea (Nash et al., 2012b). Weed and corn heights were shorter with AA and side dressed UAN which is likely due to the delayed availability of N. This effect was observed to a lesser extent with PCU on common waterhemp heights in 2007, but not in 2006.

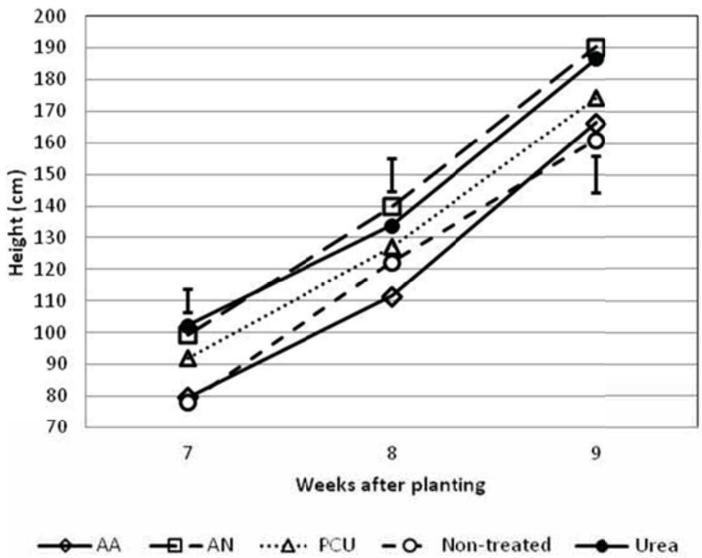

Figure 4. Corn height response to nitrogen fertilizer sources (preplant nitrogen sources (AA, anhydrous ammonia; AN, ammonium nitrate; NFC, non-fertilized control; PCU, polymer-coated urea; and urea) 7, 8, and 9 weeks after planting in the weed-free control. Data were combined over years (2006 and 2007). Vertical bars represent the standard error

3.4 Weed Control

At physiological maturity, an interaction between weed management systems and N source occurred for total weed dry weights, but no significant 3-way (year*N source*weed management system) interaction was detected (P = 0.83). Residual and sequential weed management programs helped reduce overall weed biomass, but it was the N source that affected total weed biomass (Table 2). All weed management systems showed greater than 90% weed control 28 days after treatment (visual observation), and total dry weights were less than the non-treated control. Total weed biomass was composed primarily of late-emerging common waterhemp and giant foxtail, and to a lesser extent of common lambsquarters.

Table 2. Total weed dry weight interaction between weed management system and nitrogen source at physiological maturity, and corn grain yield response to weed management systems

Weed management system[§]	Total weed dry weight[†]						Yield[‡]	
	NFC	Urea	AA	AN	PCU	UAN	2006	2007
	g m^{-2}						Mg ha^{-1}	
Non-treated	10.2	12.4	18.7	12.1	21.2	15.9	2.16	10.04
Glyphosate (POST) fb glyphosate (POST)	3.1	0.7	3.4	2.7	10.3	3.1	6.34	12.98
Atrazine (PRE) fb glyphosate (POST)	0.8	2.9	4.2	0.7	2.6	1.9	7.08	12.75
Atrazine + glyphosate (POST)	2.3	1.1	4.3	0.5	2.4	1.6	6.54	12.92
Atrazine + dimethenamid-*P* premix (PRE)	5.2	1.2	9.4	4.2	2.7	2.8	6.78	12.43
Atrazine + dimethenamid-*P* premix + glyphosate (POST)	0.4	0.1	1.3	0.7	2.2	1.5	6.51	12.65
Atrazine + *S*-metolachlor + mesotrione premix (POST)	4.4	3.7	1.5	3.6	2.6	3.5	7.25	11.98
Weed-free	0	0	0	0	0	0	7.24	13.53
LSD (*P* = 0.01)	--------------------- 4.0 ---------------------						-------1.63-------	

[†]Data were combined over years (2006 and 2007).

[‡]Data were combined over nitrogen sources.

[§]Abbreviations: AA, anhydrous ammonia; AN, ammonium nitrate; fb, followed by; NFC, non-fertilized control; PCU, polymer-coated urea; POST, postemergence; PRE, preemergence; and UAN, urea ammonium nitrate.

Differences in dry weights of individual weed species were due primarily to an interaction between year and weed management system (Table 3). Common waterhemp and giant foxtail dry weights were greater in 2007 than in 2006, probably because of more favorable overall growing conditions. However, common lambsquarters dry weights were greater in 2006 than in 2007 (data not presented). Individual weed control differences among management systems were due mainly to differences in application timing of the residual herbicide as a preemergence only (atrazine+dimethenamid-*P*) treatment compared to a sequential application of herbicides. Overall weed control differences were evident within a weed management system depending on the N source at physiological maturity (Table 2). The N source significantly affected common waterhemp dry weights (P = 0.0098) (Table 3). In a greenhouse experiment, redroot pigweed (*Amaranthus retroflexus* L.) was more competitive than corn under high NO_3-N levels (Teyker et al., 1991). Anhydrous ammonia, PCU, and side dressed UAN had common waterhemp dry weights that were 40 to 75% greater than the non-fertilized control or urea (data not presented). This indicated that PCU, deep placement of AA, and side dressed UAN also provided N to late germinating weeds such as common waterhemp. In other corn research, preplant and topdressed AN had similar common waterhemp seed production (Harbur & Owen, 2004).

Table 3. ANOVA table of giant foxtail, common lambsquarters, and common waterhemp dry weights when corn was at physiological maturity

		Giant foxtail		Common lambsquarters		Common waterhemp	
Source	df	F-value	Pr > F	F-value	Pr > F	F-value	Pr > F
Year	1	19.17	<0.0001	4.41	0.0459	67.68	<0.0001
Year*rep	5	3.06	0.0108	0.93	0.4611	1.73	0.1293
Nitrogen (N)	5	0.70	0.6262	1.19	0.3427	3.87	0.0098
Year*N	5	0.77	0.5787	0.64	0.6685	2.19	0.0878
Year*rep*N	25	2.19	0.0015	1.39	0.1100	1.16	0.2838
Weed management system (WMS)	7	11.03	<0.0001	2.37	0.0235	34.43	<0.0001
Year*WMS	7	7.02	<0.0001	2.11	0.0443	34.32	<0.0001
N*WMS	35	1.28	0.1495	1.35	0.1022	1.41	0.0763
Year*N*WMS	35	1.12	0.3070	1.45	0.0600	1.35	0.1010

3.5 Yield

Although an interaction between N source and weed management system was detected for final weed biomass, there was no such interaction for yield (P = 0.62); therefore, main effects are presented. Similarly, no interaction was observed between green foxtail [*Setaria viridis* (L.) Beauv.] density and N rates (Cathcart & Swanton, 2004), but in other research the critical period for weed control was affected by management of N rate (Evans et al., 2003a). A significant ($P < 0.0001$) two-way interaction (year* weed management system) for grain yield was observed. Grain yields averaged 6.14 Mg ha^{-1} greater in 2007 than in 2006 (Table 2), probably due to differences in precipitation distribution during the summer between years (Figure 1). Grain yields for all weed management treatments were similar to the weed-free control (Table 2). Weed management systems increased grain yield 1.65 to 5.09 Mg ha^{-1} in 2006 and 2007 compared to the non-treated control. Effective weed management systems that are based on recommended rates and timings (Table 1) provide no-till farmers with flexible management options regardless of the N fertilizer source.

All N sources increased yield compared to the non-fertilized control (Figure 5). Side dressed UAN had the highest overall yield (10.5 Mg ha^{-1}), which was similar to AA, AN, and PCU. A preemergence application of PCU and side dressed UAN increased yields 1.33 and 2.21 Mg ha^{-1} greater than urea, respectively. This was similar to other research evaluating anhydrous ammonia and PCU on claypan soils (Noellsch et al., 2009; Nash et al., 2013), but it differed from studies showing greater yield loss with a delayed application of AN compared to a preemergence application when weeds were allowed to compete with corn (Harbur & Owen, 2004). In Missouri, Stecker et al. (1993) reported greater N fertilizer use efficiency and yields (0.42 Mg ha^{-1}) with AN for no-till corn than with surface-applied urea. Since in both study years common waterhemp and lambsquarters heights were among the tallest with PCU 6 to 7 WAP, it may be assumed that greater corn yields with PCU than with urea did not stem from reduced weed growth and N uptake. This yield increase may have come from reduced environmental N loss and greater available N for corn uptake. This is counter to a four-year, no-till canola study on well drained soils in the semiarid Canadian prairies. The study found that PCU was generally effective in reducing weed biomass, but it only increased yields in 4 of 20 site-years compared to urea (Blackshaw et al., 2011). Contrasting responses among the studies presumably are due to differences in crops, soils, and climate.

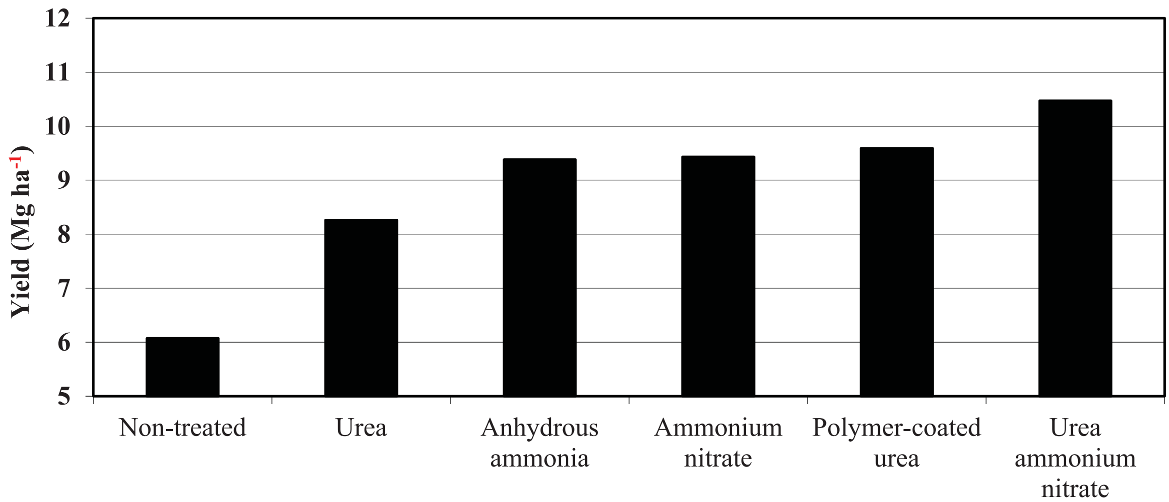

Figure 5. Corn grain yield response to nitrogen sources applied at 168 kg N ha^{-1}. Data were combined over years (2006 and 2007) and weed management systems. LSD (P = 0.01) is 1.30 Mg ha^{-1}

4. Conclusions

Small-seeded broadleaf weed heights responded differently to PCU and AA during the two years of this research. This indicated that N source might affect the critical period for weed control in a no-till production system. Corn heights were greater with AN and urea compared to PCU, AA, and side dressed UAN 7 to 9 WAP, indicating that N source could affect canopy development of no-till corn. The N fertilizer source and weed management system affected total weed biomass (giant foxtail, common waterhemp, and common lambsquarters) at physiological maturity of corn; however, these factors showed no interactive effect on corn grain yields. An early postemergence application of atrazine + dimethenamid-*P* + glyphosate reduced total weed biomass 86% and 92% compared to atrazine + dimethenamid-*P* applied preemergence following AA and the non-fertilized control, respectively. A two-pass postemergence system (glyphosate followed by glyphosate) had 74 to 79% greater weed biomass compared to residual systems when following a PCU application. All weed management systems increased yield 1.5 to 5.09 Mg ha^{-1} compared to the non-treated control, and no differences appeared among weed management systems. In this region, AA, broadcast PCU, and side dressed UAN are recommended over broadcast urea for no-till corn production. This study indicates that no-till corn farmers have several flexible options for N management and effective weed management systems that are based on label recommendations for weed heights and effective rates.

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Expansion of Eucalyptus Woodlots in the Fertile Soils of the Highlands of Ethiopia: Could It Be a Treat on Future Cropland Use?

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Abstract

A study was conducted to assess the effect of land use change from eucalyptus to cropland on soil physico-chemical properties and perceptions of farmers in Koga irrigation area, Amhara Region. Soil samples were taken from 4 sites of three land uses (eucalyptus woodlots, cropland, and eucalyptus land use changed to cropping) and at 0-20, 20-40 and 40-60 cm depths. The three depths were used for analysis of soil chemical properties, whereas the first two depths for physical properties. Furthermore, randomly selected 15 farmers were interviewed for their perception on the state of soil fertility and crop yield conditions on lands that were recently changed from eucalyptus to cropland. The result showed that except for available P, sampled plots that were changed from eucalyptus to cropland were found better in soil chemical properties (pH, N, CEC) and SOM contents as compared to croplands. As compared to the other two land uses, total N was found larger at eucalyptus woodlots. Regarding soil physical properties (bulk density and texture), little or no difference was recorded among the different land use types. On top of that, farmers perceived that plots that were under eucalyptus have better fertility, require less nitrogen fertilizer and crops perform well compared to plots that are contineously under cropping. Thus, results of this study confirmed that changing land use from eucalyptus to cropland is possible without detrimental effect on soil properties and without affecting productivity of lands to raise crops.

Keywords: eucalyptus, land use, land use change, Koga, Mecha District, soil physico-chemical property

1. Introduction

In Ethiopia, each year about 80,000 ha of natural forests have been converted to farmland, while 50,000 ha of woodlands removed for charcoal production and farmland expansion; and 30,000 ha of woodland, thickets, and bushes cleared for fuel wood production (UNDP/ World Bank, 1988 cited in Tola, 2009). Ethiopia has a sad story on its forest resources. Both Emperor Kaleb and Minilik II were very concerned on the forest resources and Emperor Menelik, assisted by his French technical advisor, introduced fast-growing tree species from southern Europe (Portugal, Italy, Greece, etc.), whereas later on Eucalyptus is assumed introduced from Australia that made up of several species. Eucalyptus was first introduced with the objectives of meeting ever-increasing demand for construction poles and firewood in Addis Ababa, the seat of King Menelik II (Yitebitu, 2010; Amare, 2010).

The radius of Eucalyptus growing from the capital city - Addis Ababa continued to increase during the first decade. The next stage of Eucalyptus growing outside Addis Ababa was by missionaries in Ghimbi, DebreTabor, and Harar. Later still, especially after the 50s, Eucalyptus growing moved to rural areas from these first nodes, being planted first in urban areas, the homesteads and eventually on agricultural lands by farmers and urban dwellers (Amare, 2010).

Today, while the debates among experts continue on its alleged ecological disadvantages, eucalyptus plantation is showing an amazing expansion in Amhara Region in particular and in Ethiopia in general by smallholder farmers. At the moment, Ethiopia has the largest area of Eucalyptus plantations (more than 0.5 million ha) (Amare, 2010) in East Africa and is one of the 10 pioneer countries that introduced the eucalyptus trees (Gessesse & Tekilu, 2011).

The preference to eucalypt by small holder farmers is due to a number of advantages gained from the tree. The tree species are preferred more than others due to their fast-growth, coppicing ability, easy silvicultural management, poorly palatable to animals, the demand for its wood products with reasonable prices, and their adaptations to a wide range of ecological conditions (Yitebitu, 2010; Mulugeta, 2010).

Even if it is claimed for its ecological disadvantage, in Mecha district fertile croplands have been converted to eucalyptus wood lots each year for two main reasons; its attractive economic return with minimal labor and capital inputs, and fear of crop yield reduction at the adjacent or neighboring eucalyptus woodlots to crop farms. In Ethiopian, still natural forests have been converted to croplands and croplands to eucalyptus woodlots in small land holdings, however, unless for a special reason, there is no land use change from eucalyptus to cropland. This might be due to the long existing fear of the alleged negative impacts of eucalyptus to soil physico-chemical properties that could cause reduction on crop yields.

Figure 1. Eucalyptus woodlot from one of the sampling site (Left, Google earth image in 2009) before excavation and after excavation (right, Google earth image in 2011)

Even if a wealth of information is available on eucalyptus, critics on the effect of eucalyptus on soil and water depletion, allelopathic effect to nearby plants and the difficulty on the future land use change to crop use are still debating. The authors were seriously questioned on what would be the fate of those fertile croplands that have been changed to eucalyptus if the need arises to change back to cropping after four or five tree harvest. Fortunately, very recently, a land use conversion was made from eucalyptus woodlots to irrigated cropping at the Koga irrigation scheme in the Amhara Region where the objective of this study could be addressed. Even though farmers in the area are much interested in continuing with eucalyptus plantation, the Koga irrigation scheme is entirely dedicated for production of food crops so as to meet part of the national food security objectives. Therefore, this study is aimed at meeting the objectives on investigating the effects of land use change from eucalyptus to cropland on soil physico-chemical properties and on evaluating the farmers' opinion on land productivity problems faced due to the present land use change. Figure 1 shows the location of the Koga irrigation scheme and the excavation process on the eucalyptus woodlots that changed to cropland.

2. Materials and Methods

2.1 Description of the Study Area

Koga irrigation scheme is situated in Mecha district, West Gojam Zone, Amhara Regional State of Ethiopia close to Merawi town - the center of the district, which lies about 35 km southwest of the regional capital Bahir Dar. Mecha district is located at 11^o 10' N to 11^o 25' N latitude and 37^o 02' E to 37^o 17' E longitude at an altitude ranging from 1,900 to 3,200 masl. The mean annual rainfall at Merawi town is 1589 mm and mean temperature is 16-20^{o}C (Nigusssie & Yared, 2010), and is subject to the inter-tropical convergence zone. This leads to a single rainy season during the months between June/July to September/October allowing for only one rain fed cropping season, referred to as *Meher* in Ethiopia. The dominant soil type in the scheme is Nitisols. Koga irrigation scheme is designed to supply irrigation water for more than 7,000 ha of land.

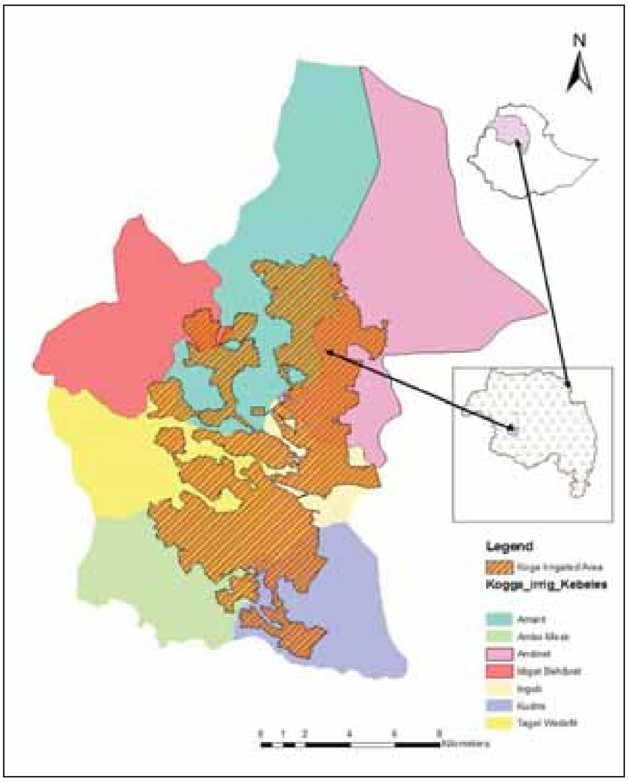

Figure 2. Koga irrigation scheme overlaid on the Kebele Administrations where the irrigation scheme found

2.2 Soil Sampling

In this study, three land use types: 1) cultivated land, 2) Eucalyptus plantation 3) eucalyptus woodlots changed to croplands were selected. For each land use, four sites were selected randomly (considered as replications), for soil sampling. Soil samples were taken from 0-20cm and 20-40cm for bulk density (24 soil samples) and 0-20cm, 20-40cm and 40-60cm for other parameters (36 soil samples); a total of 60 soil samples were taken and used for the study.

2.3 Soil Preparations and Analysis

The samples were oven-dried at 105ºC for bulk density determination. Soil-water content was determined by gravimetrically. For chemical analysis and textural analysis soil samples were air dried under shade, ground using pestle & mortar and sieved to pass through 2 mm. Particle size analysis was determined by hydrometic method as described by Sahilemedhin and Taye (2000). The soil pH was determined using glass electrode pH meter in 1:2.5 soil to water suspension following the procedure outlined by Sahlemedihn and Taye (2000). The organic carbon content was analyzed by wet digestion method using the Walkley and Black procedure (Nelson & Sommers, 1982) and converted to soil organic matter by multiplying it by the factor of 1.724. The total nitrogen was determined by Kjeldahl method (Bremner & Mulvaney, 1982), Available phosphorus was determined according to Bray II method (Sahilemedhin & Taye, 2000).

2.4 Farmer's Perception on the Effect of Land Use Changes from Eucalyptus Woodlot to Cropland.

Farmers perception on eucalyptus land use before excavation and after excavation/changed to cropland and its productivity as compared to croplands that were constantly used for long years was assessed through semi structured interviews made in 4 Kebbeles (the smallest administrative unit) and different irrigation blocks. A purposive random sampling was made for 15 farmers who have farmlands changed from eucalyptus to cropland use. The respondent answer summarized in tabular form, analyzed in descriptive statistics and incorporated as a part of research result in this paper.

2.5 Statistical Data Analysis

The impact of independent variables such as land use on the dependent variables (soil properties) was statistically tested. Analysis of variance (ANOVA) was carried out using SAS software (SAS, 2008). For variables showing statistically significant difference between treatments (p<0.05), further analysis of mean separation was carried out using Duncan's Multiple Range Test (DMRT) at 5% probability.

3. Results and Discussion

3.1 Soil Physical Properties

Bulk density (g cm^{-3}) showed no significant difference between the different land uses for the surface 0-20 cm and 20-40 cm depths (Table 1). In terms of absolute value, however, cropland had the highest bulk density of 1.11 g cm^{-3} in 0-20 cm and 1.24 g cm^{-3} in 20-40 cm depth of the soil. Soil under eucalyptus woodlots and land use changed from eucalyptus to cropland had similar bulk density, both exhibited lower bulk densities as compared to cropland in both depths (Table 1). This result is in agreement with Fekadu et al. (2012) who reported similar results for Wondo Gent area in Ethiopia as bulk density didn't show significant difference between the different land uses and soil depths with the range of (0.93-1.07 g cm^{-3}). Again Tilashwork et al. (2012) also found that in Koga irrigation watershed, soils bulk densities at all depths and distances from *Eucalyptus camaldulensis* and *Croton macrostachyus* stands are low and ranged from 1.0 to 1.1 g cm^{-3}. The lower bulk density in crop lands as compared to eucalyptus plantation also reported by (Yihenew & Getachew, 2013). The lower bulk density under eucalyptus may be due to organic matter accumulation and les trampling by livestock. As noted by Kimmins (1997), structure, texture, and porosity of soils, together with their organic matter content, combined to together to determine bulk density of a soil. All land utilization types are in good condition on their bulk density. For good plant growth, bulk densities need to be below 1.4 g cm^{-3} for clays soils (Miller & Donahue, 1997).

Table 1. Mean of bulk density (g cm^{-3}) in the soil layer of 0-20 and 20-40 cm and soil textural class in 0-20, 20-40 and 40-60 cm depths and across different land uses

LUT	Depth (cm)	Bulk Density (g cm^{-3})	Soil texture %			Textural class
			Sand	Silt	Clay	
Eucalyptus	0-20	1.07	18.28	26.32	55.40	Clay
	20-40	1.17	12.28	21.32	66.40	Clay
	40-60	-	11.92	16.41	71.67	Clay
Cropland	0-20	1.11	17.56	25.00	57.44	Clay
	20-40	1.24	16.28	12.32	71.40	Clay
	40-60	-	13.36	14.00	72.64	Clay
Recently converted cropland (previous eucalyptus)	0-20	1.08	18.28	27.32	54.40	Clay
	20-40	1.16	13.14	21.23	65.63	Clay
	40-60		13.9	14.2	71.9	Clay

The soil texture analysis result showed that sand and clay didn't show significant difference (P>0.05) across land uses whereas, silt texture showed significant difference (P<0.01). Similarly, sand texture showed significant difference (P<0.05), whereas silt and clay (P<0.01) across depth. Sand and silt texture decreased down a depth whereas clay increased (Figure 3).

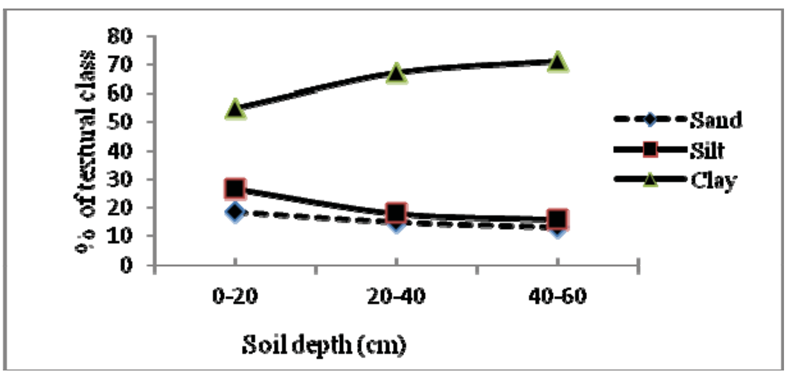

Figure 3. Percent of soil texture across soil depths

The soil textural analysis result against the land use and soil depths are presented in Table 1. Clay texture was dominated all over the land uses and depths ranged from 54.75 to 73.11%. The silt fraction was ranged from 12.32% in croplands with the depth of 20-40 cm and it was 27.32% in 0-20 cm depth under land use change from eucalyptus to cropland. Nevertheless, sand and silt decreases down a depth whereas clay content increases down a depth in all the three land uses. All land uses and depths of the soils in this study were fund dominated by the clay fraction. In areas like Koga, where the soil texture is dominated by clay, lower bulk density is reliable for seed germination and crop growth. Clay texture has good CEC as compared to other textural classes. In general, fine-textured soils hold more water and plant nutrients and thus require less frequent applications of water, lime, and fertilizer (Daniels & Haering, 2011). Soils with high clay content (more than 40%), however, hold less plant available water than loamy soils. Fine textured soils have a narrower range of moisture conditions under which they can be worked satisfactorily than sandy soils. Soils with silt and clay content may puddle or form surface crusts after rains, impeding seedling emergence. High clay soils often break up into large clods when worked in either too dry or too wet conditions.

3.2 Soil Chemical Properties

3.2.1 Soil pH and Exchangeable Acidity

Some important soil chemical properties of the soils under different land uses were significantly different and some were not. For instance, soil pH for the three land use types showed statistically significant different ($P<0.05$), whereas exchangeable acidity didn't show significant difference. All land uses exhibited acidity, although the soil under Eucalyptus showed even a relatively lower pH 5.20 and higher exchangeable acidity (0.78) as compared to the land use changed from eucalyptus to crop (5.51 pH and 0.66 $cml_c\ kg^{-1}$ EA) and croplands (5.41 pH and 0.60 $cml_c\ kg^{-1}$ EA) value (Table 2). Even if, the pH of the area is recognized as acidic, the land use changed to cropland, however, showed an improvement in soil pH by 0.29 unit and a reduction of EA by 0.12 $cml_c\ kg^{-1}$ as compared to soils under eucalyptus land cover. This might be due to litter and root biomass decomposition could raise and release some cations. Robson and Abbott (1989) discussed that soils are extremely heterogeneous with large variations in pH in short distances due to root activity, decomposition of organic matter, nitrification, etc. The other reason for lower pH in eucalyptus woodlots could be high Cation uptake by the tree and removal of cations with the frequent harvest of tree. As reported by Binkley et al. (1992), intensive management, including frequent harvesting of high nutrient content aboveground biomass can lower the availability of all soil nutrients and lead to soil acidification. The third reason for low pH could be high rainfall of the area that could leach cations to deeper horizons and/or depleting the rhizosphere. As reported by Pritchett and Fisher (1987) when leaching of acid soils proceeds over time, the proportion of exchangeable Al^{3+} and H^+ ions on clay and humus increases, whereas the proportion of exchangeable Ca^{2+}, Mg^{2+} and K^+ decreases.

Our result is in line with the findings of different authors; Lalisa et al. (2010) and Yihenew and Getachew (2013) reported that in Ethiopian central highlands that soil pH is lower in wood lots/eucalyptus plantation as compared to homestead, croplands and grazing lands. Zerfu (2002) also noted that *eucalyptus globulus* plantation sites showed high level of active acidity or low pH as compared to agricultural lands.

Table 2. Mean soil chemical properties under different land uses

LUT	Sample size (N)	pH (1:2.5Water)	OM (%)	N (%)	P ppm	CEC ($cmlo_c\ kg^{-1}$)	Ex-acidity ($cmlo_c\ kg^{-1}$)
Eucalyptus	n1	5.20 b	3.21 a	0.18	14.94	21.33	0.78
Continuous Crop	n2	5.41 a	2.86 b	0.15	17.96	20.69	0.60
Recently cropland	n3	5.51 a	3.36 a	0.15	16.27	21.45	0.66
Probability	-	*	*	**	Ns	Ns	Ns
CV (%)		4.02	11.20	11.48	22.22	13.42	38.8

** significant ($P\leq0.01$), * significantly different at $P\leq0.05$ and ns denotes for not significantly different.

The pH of the soil showed significant difference ($P<0.05$) with depths. The lower pH 5.25 was found in deeper soils (40-60 cm) at permanently croplands and recently converted croplands, whereas the highest pH that was recorded in the intermediate depth (20-40 cm) at all land uses except there is about 0.14 unit lower at the eucalyptus woodlots (Figure 4).

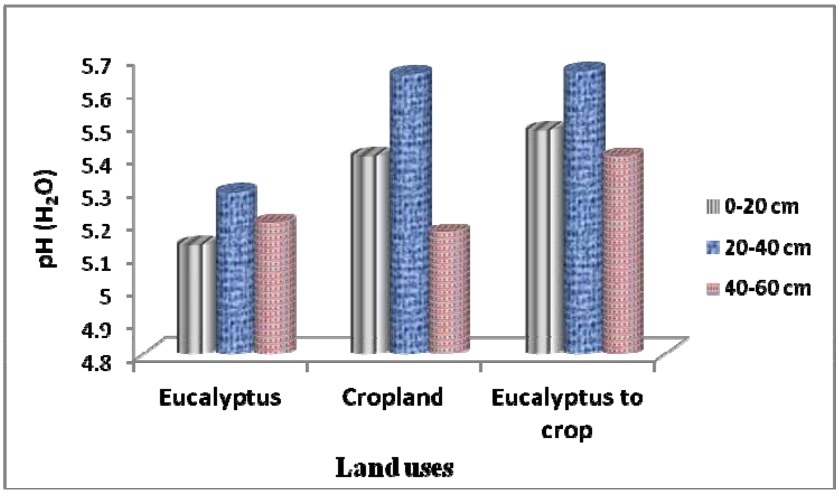

Figure 4. Soil pH as influenced by interaction of land use and soil depth

3.2.2 Organic Matter

Organic matter showed statistically significant difference ($P<0.05$) on land use types and soil depths. Land use change from eucalyptus to cropland showed higher organic matter content up to 3.36% than the two land use types (Table 2). Croplands had the lowest OM content (2.86); this could be due to a complete removal of crop residues for animal feeds and fire wood. This result is in agreement with Lalisa et al. (2010) that organic carbon is lowest in cereal fields as compared to other land uses in the central highlands of Ethiopia.

As shown in Table 3, organic matter decreased down a soil depth. Larger OM (3.65%) was found in the surface soil (0-20 cm depth) followed by 3.07% at 20-40 cm and 2.71% in the 40-60 cm depths. The highest OM up to 3.91% and 3.64% were recorded on land use changed from eucalyptus to cropland and under eucalyptus land use at 0-20 cm, respectively. However, the lowest OM (2.36%) was recorded on croplands at 40-60 cm soil depth (Figure 4).

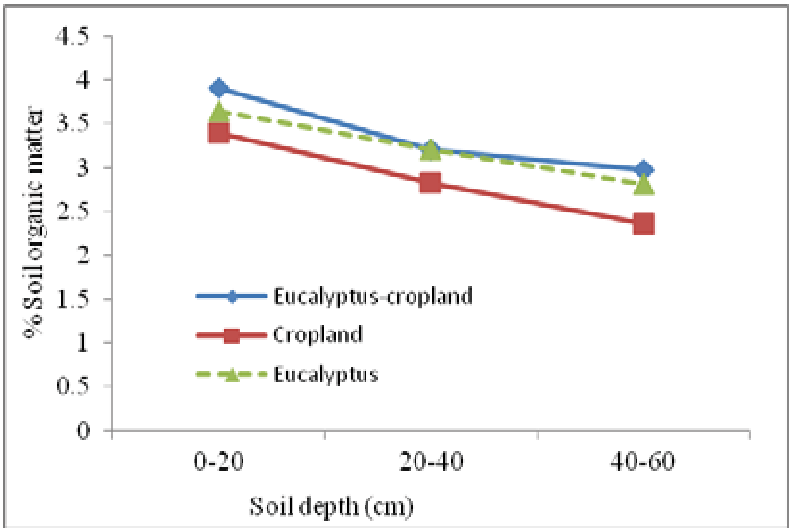

Figure 5. Soil OM distribution in different land uses and soil depths

This result is confirmed by Muluneh (2011) that soil under eucalyptus at Jufi site of Achefer district, Ethiopia showed a decrease in soil organic matter down a depth. Lelisa et al. (2010) also showed that organic carbon content of the soils at homestead, cereal farm and wood lots/eucalyptus land uses is found lower with soil depths. It is obvious that high accumulation of organic matter can be found in the surface soil where large amount of root biomass and other plant debris can be found. Trees generally improve ground cover, add organic matter to the soil, reduce the erosive impact of falling rain and improve infiltration of water into the soil (Jagger & Pender, 2000).

Table 3. Mean soil chemical properties under different soil depths

Depth	pH (1:2.5Water)	OM (%)	N (%)	Av.P (ppm)	CEC (cmol$_c$ kg^{-1})	Ex-acidity (cmol$_c$ kg^{-1})
0-20	5.34 b	3.65 a	0.16b	16.83	21.29	0.61
20-40	5.53 a	3.07 b	0.15c	16.25	21.05	0.64
40-60	5.25 b	2.71 c	0.17a	16.08	21.15	0.80
Probability	*	**	**	ns	ns	Ns
CV (%)	4.02	11.20	11.48	22.22	13.42	38.8

** significant (P≤0.01), * significantly different at P≤0.05 and ns denotes for not significantly different.

3.2.3 Total Nitrogen

Significant difference (P<0.01) in total nitrogen content of the soils were found due to LUTs and soil depths. Total nitrogen was as much as 0.18% under eucalyptus plantation, whereas it was 0.15% on both cropland and land use changed from eucalyptus to cropland (Table 2). The rise of total N under eucalyptus might be the presence of higher organic matter in the surface whereas the low N content in the croplands may be due to high decomposition rate on the plow surface and high N uptake of the cereal crops. Davidson (1993) also reported that a substantial proportion of the nutrients in a tree crop held in the foliage which is returned periodically to the soil. This nutrient turnover is responsible for improvement of soil fertility by plantations established on previously cleared sites. Maintenance of the nutrient cycle is critical to the long term productivity of soils and it is essential foliage and leaf litter are not removed from the site. Even greater benefits accrue if bark is also left behind the relative amounts of nutrients in different parts of the biomass of *E. tereticornis* in India (Davidson, 1993). Sanginga and swift (1992) reported that in Zimbabwe the Eucalyptus plot had higher NH_4 - N contents than the Miombo woodland and bare soil/croplands. Soils under Eucalyptus forests also reported as it has larger soil nitrogen due to the long period of time under tree cover and soil nitrogen mineralization could be increased under Eucalyptus forests by 11-14 ppm per year (Reversat, 1988; John et al., 2005; Muluneh, 2011). A study result by Lalisa et al. (2010), in central highlands of Ethiopia, indicated that total N was lower in cereal farm as compared to pasture land and wood lots. Even if Tilashwork et al. (2012) discussed that total N (TN) increased with distance from the eucalyptus trees, their result showed that near the Eucalyptus stand the TN was significantly larger (p ≤ 0.01) of the average TN of the field and the nitrogen content declines with increasing distance up to 5 m away from the tree. Generally higher N near eucalyptus trees might be due to the higher N in the foliage of eucalyptus and low uptake by the tree. This was discussed by Selamyhun (2004) that foliage of eucalyptus accounted 45-48% of total N from above ground parts but the mean annual nitrogen accumulation of eucalyptus ranged from 8-13 Kg N ha^{-1} y^{-1}. The other reason for high N under eucalyptus woodlots might be due to the low temperature and very limited radiation reached on the surface of the soil that leads to low volatilization of NH_3 - N.

N content was also found increased significantly with the soil depth (P<0.01). The higher total N (0.17%) was found on the 40-60 cm depth range, whereas total N content was 0.16 % and 0.15% at 0-20 cm and 20 40 cm soil depths. The interaction effect of depth with land use on total N also showed significant differences (P<0.01). Total N was very low (0.11%) in 20-40 cm soil layer at a land use where changes from eucalyptus to crop, whereas higher N up to 0.19% was found in 20-40 cm depths under eucalyptus land use followed by 0.18% on cropland at 40-60 cm soil depth. Total N increased down a depth on croplands, whereas at eucalyptus woodlots showed an increase in the middle zone (20-40 cm). Contrary to croplands, total N at 20-40 cm depth declined in a land use changed from eucalyptus to crop. This could be due to the leaching of N to lower depth and removal of the nutrient by annual crops on the surface soil. It is also known that in cereal dominated farming system, competition for nutrient is higher in the surface layer due to the root biomass on this layer is much denser than the other layers. On the other hand, higher N on surface soil under eucalyptus could be the large amount of root biomass of the tree is found in lower or sub surface horizons of the soil. Selamyhun (2004) pointed out after eight years, fine roots (<10 mm diameter) accounting for 80% of the total root mass per unit area are mostly extend laterally to less than 20 m lateral distance and 60-100 cm depth in to the adjacent cropland areas in Nitisols of the central highlands of Ethiopia.

3.2.4 Available Phosphorus

Generally, the phosphorus content of the area is very low. Available P didn't show statistically significant difference (P>0.05) due to land uses, whereas croplands had relatively better phosphorus (18.07 ppm) as compared to lands covered by eucalyptus woodlots (14.94 ppm) and land use changed from eucalyptus to cropland (16.27 ppm). The larger available P in croplands might be due to the cumulative effects of application of phosphorus containing fertilizer in the past.

Similar finding is reported by Yechale and Solomon (2011) that the amount of available p content in croplands is much larger than in forest and eucalyptus woodlots. Woldeamlak and Stroosnijder (2003) also reported that the available P content of cultivated fields is larger than forest lands, which suggests that the annual leftover of P from fertilizer application is much larger than obtained from leaf decomposition from trees. Lisanework and Michelsen (1994) explained that the lower available P content at forest lands is due to a high proportion of P is retained and immobilized by microbes. Another possible reason for low available P under eucalyptus woodlots could be due to the lower pH conditions that can permanently fix phosphorus. Under acid conditions, phosphorus is precipitated as Fe or Al phosphates of low solubility (Tisdale et al., 2002), whereby maximum availability of phosphorus generally occurs in a pH range of 6.0 to 7.0. It was also found that under eucalyptus plantation, exchangeable Al is relatively higher than the two land uses. The raise of Al may lead to Al toxicity and lowering the soil pH. In this study, land use changed from eucalyptus to cropland suggests that the effect of removing the eucalyptus woodlots and replacing this with annual crops was not negatively affect on availability of phosphorus. A similar study in tropical India showed that insignificant change in available P following deforestation (Saikh et al., 1998 as cited by Yechale & Solomon, 2011).

In general, there is evidence in the literature suggesting that eucalyptus may enhance some soil conditions, including moisture and topsoil retention, particularly on degraded or barren sites through leaf litter (Jagger & Pender, 2000). In higher plantation area like Mecha district, almost all leaf litter fall down under eucalyptus, which may pump nutrients from deeper parts of the soils through their roots to the litter that will give rise nutrients. Even after harvest some tree growers used to collect the leaves and twigs for in-situ burning before new shoots /coppice emerged. Soil nutrient levels under eucalyptus plantations can also be improved by leaving litter uncollected, adjusting spacing and mixing eucalyptus species with leguminous plants (Tesfaye, 2009). In addition, Davidson (1995) showed that eucalyptus species have a beneficial effect on soil structure and soil fertility improvement through decayed litter. Similarly, Yitebtu (2010) found that after 8 years of plantation, availability of macronutrients like phosphorous, potassium, calcium and magnesium are increased by 57%, 13%, 31% and 45%, respectively.

3.2.5 Cation Exchanging Capacity (CEC)

CEC didn't show statistical differences (P>0.05) between LUTs (Table 2). In terms of absolute value, however, land use changed from eucalyptus to cropland had relatively larger CEC (21.45 $cmol_c\ kg^{-1}$) and under eucalyptus land use, it was about 21.32 $cmol_c\ kg^{-1}$, whereas the lower CEC (20.69 $cmol_c\ kg^{-1}$) was obtained on croplands. CEC on depth and interaction of land use with depth also didn't show statistical differences. The CEC of all the land uses were very acceptable, and indicates that the soils of the area are relatively fertile. Our result is confirmed by (Lalisa et al., 2010) that CEC didn't show any significant difference among the land uses in the central highlands of Ethiopia. Yechale and Solomon (2011) also confirmed that there was no statistically significant difference between soils among the land uses in terms of the CEC that was studied in the Hare river watershed, Ethiopia.

3.3 Farmers Perception on Land Use Change from Eucalyptus to Cropland

From the 15 farmers interviewed, 11 of them appreciated eucalyptus planting on croplands is economically important than other land uses. Considering eucalyptus as an asset is also found the most important by many farmers. They believed that eucalyptus assumed as money deposited in a bank. The other benefits according to the respondents were, the labor required to produce eucalyptus is minimal. On the contrary, two respondents appreciated the cropland use due to its frequent and immediate return to investments as compared to tree growing. Only one respondent said that as there is no economical difference between crop and tree growing. Another respondent said that eucalyptus and croplands should be used according to the fertility status of the soil; for poor soil, eucalyptus is more preferable than growing it on fertile soils that are suitable for crops.

Except one, all farmers appreciated changing eucalyptus land use to cropland due to better yields obtained on such lands. Even though farmers recognized that the crop yield is better at the recently converted croplands, no one could tell the exact difference quantitatively. From the respondents, five of them recognized that fertilizer amount required for the formerly eucalyptus land use as compared to contineous croplands is somehow lower. Four farmers were replied as they reduced the fertilize use by a quarter. Although most farmers perceived that eucalyptus land changed to cropland can give better yield than the continuously cropped lands, they were not interested to change their eucalyptus land to cropland for a simple reason that labor and input requirements for producing food grains are cumbersome.

The other important thing as of many farmers and intellectuals, fourteen of respondents didn't assume that eucalyptus could be for cropland. They considered eucalyptus land use can only be used for eucalyptus otherwise it is a waste land; this is because they have experience as there is limited vegetation undergrowth in eucalyptus woodlots and the detrimental effect on the adjacent crops (shading effect). They also assumed that eucalyptus can deteriorate the soil and this deteriorated soil cannot be re-used for cropping after excavation of the eucalyptus.

Finally for the question "what if the decision is left for you whether you change the land use to crop or left as it is despite the irrigation water available to your farm"; nine of the respondents (60%) replied as they will change to cropping, four responded (26.67%) will leave eucalyptus as it is, whereas two (13.33%) respondents said they will excavate according to the soil fertility status for cropping. However, many agreed that whenever there is labor scarcity for the routine irrigation activity, prefer eucalyptus production than cropping. On the contrary, in case labor is not a limit, they preferred changing the land use from eucalyptus to cropland since irrigation farming pays much better than tree cropping.

4. Conclusions

The existing assumption on the environmental consequences of eucalyptus plantation is that the tree is detrimental to soil fertility and ground water, whereby future land use change to cropping is unlikely. In attesting this a study was conducted to determine the effects of land use change from eucalyptus to cropland on soil physico-chemical properties and to assess farmers' perception and opinion on the actual problems faced due to changing their land use from eucalyptus to growing food crops.

Results of the soil chemical properties indicate that except available P all the analyzed parameters at the land use changed from eucalyptus to cropland (in the last three years) were found better than the lands permanently under food crops. This newly changed land use is also better in its soil acidity and exchangeable acidity status than the lands under eucalyptus woodlots. The larger P on existing croplands could be the carry-over effect of the phosphorus fertilizers that could have been applied for the annual crops.

Farmers in the study area recognized that land use changed from eucalyptus to cropland had a yield advantage than from the plots under contineously under cropping and again the former land use requires lower amount of nitrogen fertilizer. Therefore, it is possible to conclude that changing land uses from eucalyptus to cropland has no detrimental effect on soil fertility and productivity of crops. Thus, the result confirmed that one can shift his/her eucalyptus woodlot to crop production as the need arises. However, mmonitoring the soil physico-chemical properties due to land use change from eucalyptus to cropland need to be accompanied by agronomic trials so as to depict the soil conditioning effect of tree cropping.

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Seasonal Vegetative Growth in Genotypes of *Coffea canephora*, as Related to Climatic Factors

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Abstract

Knowledge about the seasonality of different genotypes of *Coffea canephora* is an important tool for this crop management, particularly with regard to irrigation and fertilisation issues. This study was conducted in Espírito Santo, Brazil and aimed at to evaluate the seasonal vegetative growth in genotypes of *C. canephora*, as related to climatic factors, based on the growth of groups of orthotropic and plagiotropic branches with different ages. Three groups of plagiotropic branches and one group of orthotropic branches of 14 genotypes (Ipiranga and 13 that belonged to the variety Vitória) were selected and marked to followed along the one-year experiment. Three-year-old plants were cultivated under full-sun conditions, with a spacing of 3 m between rows and 1 m between plants. The growth rates of the orthotropic and plagiotropic branches differed among the genotypes and underwent seasonal variation during the entire year, with high correlations to the air temperature. Under the natural experimental conditions, the growth rate of the branches decreased when the minimum air temperatures were below 17.2ºC for most of the genotypes studied. The plagiotropic branches presented lower vegetative growth, mainly for the coffee berries, compared to the younger branches. Presumably, the genotypes of *C. canephora* demanded more nutrients for growth between mid-September and the second week of May.

Keywords: Robusta coffee, different genotypes, temperature, plagiotropic branches

1. Introduction

The genus *Coffea* has more than 124 species, among which *C. arabica* L. and *C. canephora* Pierre ex A. Froehner (Davis et al., 2011) are responsible for the yield of about 99% of traded coffee bean. In recent years, the global production of coffee surpassed 144 million bags (Ico, 2013). Brazil is the largest coffee producer and exporter worldwide. In the 2012 the production of *C. arabica* (Arabica coffee type) and *C. canephora* (Robusta coffee type) reached approximately 38.4 and 12.5 million bags (each bag of 60 kg), respectively, occupying an area of 2.27 million hectares (Conab, 2013), being exported a total of about 32 million bags in 2011 (Ico, 2013). Although *C. arabica* is the most cultivated species, *C. canephora* is increasing its importance, worldwide. The same is occurring in Brazil, which has most of its *C. canephora* production in the State of Espírito Santo, which in the 2012 was responsible for 8.49 million bags (Conab, 2013).

In *C. canephora*, inflorescences (glomeruli) are formed from ovules (one glomerulus for each ovule) randomly located in the side-leaf axils formed during the growing season of the current year. Therefore, flowering closely depends on the growth of plagiotropic branches. In coffee, reproductive development begins with gemmation, or flowering, followed by the formation of fruitlets and berry expansion until reaching their normal size. The berry formation and ripening stages are then observed (A. P. Camargo & M. B. P. Camargo, 2001; Pezzopane et al., 2003; Moraes et al., 2008; Petek et al., 2009).

The growth pattern of Arabica coffee is modified by increasing the photoperiod to 14 hours. Fruit removal does not prevent decreased growth rates of branches and leaves, though these organs are larger in coffee trees without fruits. Furthermore, fluctuations in the potential photosynthetic rates do not explain growth variations, and decreases in these rates may be related to biochemical resistance in the chloroplasts. Increased stomatal resistance in the afternoon coincides with drastic reductions in the growth of branches and leaf area. Moreover, the growth of leaves and orthotropic and plagiotropic branches follows the curves of minimum, average, and maximum temperatures (Silva et al., 2004; Amaral et al., 2006).

In coffee, low temperatures (below the range of 13-17 ºC, depending on the authors and genotypes) and pronounced water deficit (-3 MPa) affect various components of the photosynthetic process, as it reduces the stomatal conductance, net photosynthesis, photochemical efficiency of photosystem II, thylakoid electron transport, enzyme activity and carbon metabolism as a whole. Low temperatures also affect the composition and structure of photosynthetic pigment complexes and of the lipid matrix of cell membranes, particularly in the chloroplast, although to different extent among genotype and species (Campos et al., 2003; Ramalho et al., 2003; Silva et al., 2004, Praxedes et al., 2006; Partelli et al., 2009, 2011; Batista-Santos et al., 2011). Such changes may reflect impairments or damages, but in some cases are responses towards plant cold acclimation. Such acclimation ability on coffee genotypes seems to greatly rely on the control of antioxidative conditions, frequently linked to a lower photochemical use of energy through photosynthesis, as observed to happen under low temperatures (Ramalho et al., 2003; Fortunato et al., 2010), water deficit (Ramalho et al., 1998; Lima et al., 2002) and high irradiance and N-deficiency (Ramalho et al., 1998) stresses. Therefore, these changes would configure distinct morphological and physiological acclimation traits in *Coffea* spp.

When cultivated at low temperatures (below 17ºC), *C. canephora* presents marked decreases in growth (Libardi et al., 1998; Partelli et al., 2010) and photosynthesis (Ramalho et al., 2003; Batista et al., 2011), with negative impact on yield. The climate parameters for the zoning of *C. canephora* varieties are based on the region of origin. Thus, understanding the seasonal characteristics of the vegetative growth of *C. canephora* in the most important Espírito Santo region is a decisive tool for crop management, mainly irrigation, and the planning of crop fertilisation programmes. Because there are few studies related to *C. canephora* vegetative growth and the effects of climate, the present work aimed to evaluate the vegetative growth rates of the branches of different genotypes and relate this growth pattern to climatic factors. This analysis was based on the seasonal growth of groups of orthotropic and plagiotropic branches of different ages, in Nova Venezia, northern Espírito Santo.

2. Material and Methods

The experiment was conducted in the municipality of Nova Venezia, northern Espírito Santo, Brazil, located approximately at 18°43'46"South, 40°23'10"North, with an average elevation of 100 m. According to the Köppen classification, the climate is Aw, tropical with a dry season. During the experiment, the average values of temperature, relative air humidity, and rainfall were collected by the automatic weather station located at 3.8 km from the study area, at 154 m of elevation.

The evaluated *C. canephora* plants were three years old and grown under full sun, with a spacing of 3 m between rows and 1 m between plants within the row. The soil is classified as cohesive Red-Yellow Latosol, with dystrophic saturation, low CTC, clayey, and undulated relief (Embrapa, 2006). The following values for soil properties were obtained: pH (water) of 5.41; 6.1 mg dm^{-3} P; 66 mg dm^{-3} K; 1.35 $cmol_c$ dm^{-3} Ca; 0.78 $cmol_c$ dm^{-3} Mg; 7.0 mg dm^{-3} S-SO_4; 0.2 mg dm^{-3}B; 0.4 mg dm^{-3} Cu; 36,8 mg dm^{-3} Fe; 21.0 mg dm^{-3} Mn; and 3.2 mg dm^{-3} Zn in the 0.00-0.20-m layer. The plants were properly managed and irrigated during the experiment.

The experiment consisted of 14 genotypes: plants belonging to the variety Vitória 'Incaper-8142' of *C. canephora* (1V, 2V, 3V, 4V, 5V, 6V, 7V, 8V, 9V, 10V, 11V, 12V, and 13V) (Fonseca et al., 2004) and the super-late cultivar (Ipiranga-501). Each plot consisted of five randomly marked plants, with their branches marked on 09/10/2010. Four groups of branches were used: orthotropic (Ortho), old plagiotropic with berries (PlagVCC), old plagiotropic without berries (PlagVSC), and new plagiotropic (PlagN). The PlagVCC branch was randomly marked by observing the age and number of productive buds. The PlagVSC branch was marked as the last plagiotropic branch grown in the orthotropic branch. The orthotropic branch was marked from the base of the PlagVSC branch. On 29/01/2011, one plagiotropic branch was selected and marked on each plant. Measurements were obtained at an average interval of 15 days for one year (until 08/10/2011).

From those measurements, the daily rate of vegetative growth of the different treatments and groups of plagiotropic and orthotropic branches was calculated. The data for vegetative growth were subjected to an analysis of variance ($P<0.05$) in a completely randomised design. The association between vegetative growth and low temperature was also assessed. The increases are discussed and related to the climatic data and crop irrigation.

3. Results and Discussion

Within the experimental period, the minimum and average temperatures (Figure 1A) presented the lowest average values from May 16 to September 24 (autumn-winter), which characterised a period of conditions adverse to the growth of orthotropic and plagiotropic branches (Libardi et al., 1998; Partelli et al., 2010). In fact, our data agree with those results, as lower growth was observed during this period in almost all of the genotypes studied (Figure 2), confirming that *C. canephora* shows reduced growth during winter (Libardi et al., 1998; Partelli et al., 2010).

Coffee plants grown under low positive temperatures, superimposed to severe water deficiency, suffer several types of impairments and damages with regard to the photosynthetic process, leading to reductions, stomatal conductance, net photosynthesis, photochemical efficiency of photosystem II, thylakoid electron transport, enzymatic activity, and carbon metabolism and, ultimately, in vegetative growth, although with different sensitivities amongst genotypes and species (Ramalho et al., 2003, Silva et al., 2004, Partelli et al., 2009, 2011, Batista-Santos et al., 2011), with *C. canephora* being more sensitive than *C. arabica*.

Reduced growth (Figure 2) was notable when the average minimum temperature was below *ca* 19 ºC (V4, V5, V6, V7, V8, V9, V10, V12, Ipiranga), by the end of March, but decreased growth in some of the branches were found already at *ca*. 21 ºC (V1, V2, V3, V11, V13). Therefore in some cases, growth decreases may begin by in the end of February (Figure 1A), at least for some of the kind of studied branches. A marked decline in growth was observed in most genotypes (V1, V2, V3, V4, V5, V9, V10, V13, Ipiranga) (Figure 2), whereas, although with significant growth reductions, were able to maintain continuous growth (V6, V7, V11, V12). An exception was observed for genotype V8, which maintained high growth rates for most branch groups, especially after the middle of June.

The maintenance of growth in some *C. canephora* genotypes may be associated with mechanisms of tolerance to low temperatures and drought, including the postponement of dehydration to maintain whole-tree photosynthesis, xanthophyll cycle efficiency, increased antioxidant enzyme activity (Fortunato et al., 2010; Silva et al., 2013), and altered leaf lipid and sugar class composition, which might differ among these *C. canephora* genotypes, as found in *Coffea* spp. (Campos et al., 2003; Partelli et al., 2009, 2011). However, to these differences could have contributed the differences in the maturation cycle, as the varieties studied here included those with early, average, late, and super-late maturation cycles (Fonseca et al., 2004).

In general, the highest vegetative growth rates was observed from October 2010 (the start of the measurements) until May 2011 and, subsequently, from mid-September 2011 when most genotypes resumed growth (Figure 2). Thus, most *C. canephora* genotypes may require more nutrients for growth from mid-September to the second week of May due to their higher growth rates.

A slight decrease in the growth rate was observed in summer/autumn (Figure 1), which cannot be attributed to conditions of low minimum air temperature. These punctuated decreases in the growth rate of some genotypes may be associated with a severe attack of *Hemileia vastatrix* that occurred in some clones, high maximum air temperatures (above 34ºC, with some days exceeding 38°C), and the water deficit observed in January and February (Figure 1AB). It must be noted that decreases in the growth rate values varied according to the genotype studied, as *C. canephora* responds differently to soil water deficit (DaMatta et al., 2003; Praxedes et al., 2006).

Considering the several branch groups, the younger plagiotropic branches without berries (PlagN), followed by the old plagiotropic branches without berries (PlagVSC), generally showed higher growth than the orthotropic branches (Ortho) and old plagiotropic branches with berries (PlagVCC), which showed almost no growth before the harvest (Figure 2), what agrees with previous data of our group (Partelli et al., 2010). It is possible that the lowest growth of the old branches may be associated with the genetics of coffee plants and the high demand of carbohydrates and nutrients required to produce the fruits. In addition to the large quantities of carbohydrates required during the expansion phase, the fruits of coffee trees also require substantial amounts of both potassium and nitrogen, which are reallocated from leaves and trunks to the fruit (Partelli & Marré, 2012). Indeed, decreases in the nitrogen content may impair photosynthesis, thereby contributing to the creation of excess energy in the photosynthetic apparatus (DaMatta et al., 2002), which may ultimately lead to oxidative stress and cell damage (Fortunato et al., 2010). Therefore, more than one group of plagiotropic branches should be marked for the estimation of seasonal coffee growth.

The regression analyses between the growth of branches and all the environmental parameters were not significant when the entire experimental period was considered (data not shown). However, a more detailed analysis considering only the period of decreasing temperature allow to find significant correlations with the minimum air temperature (Figure 3). The best adjustment was obtained using the average minimum temperatures observed from 26/03/2011 to 10/09/2011, with R^2 = 0.87 for the orthotropic branches, R^2 = 0.91 for the old plagiotropic branches without berries, and R^2 = 0.92 for the new plagiotropic branches (Figure 3). These results confirm that low air temperature is an important variable reducing the growth of *C. canephora* in the autumn/winter season.

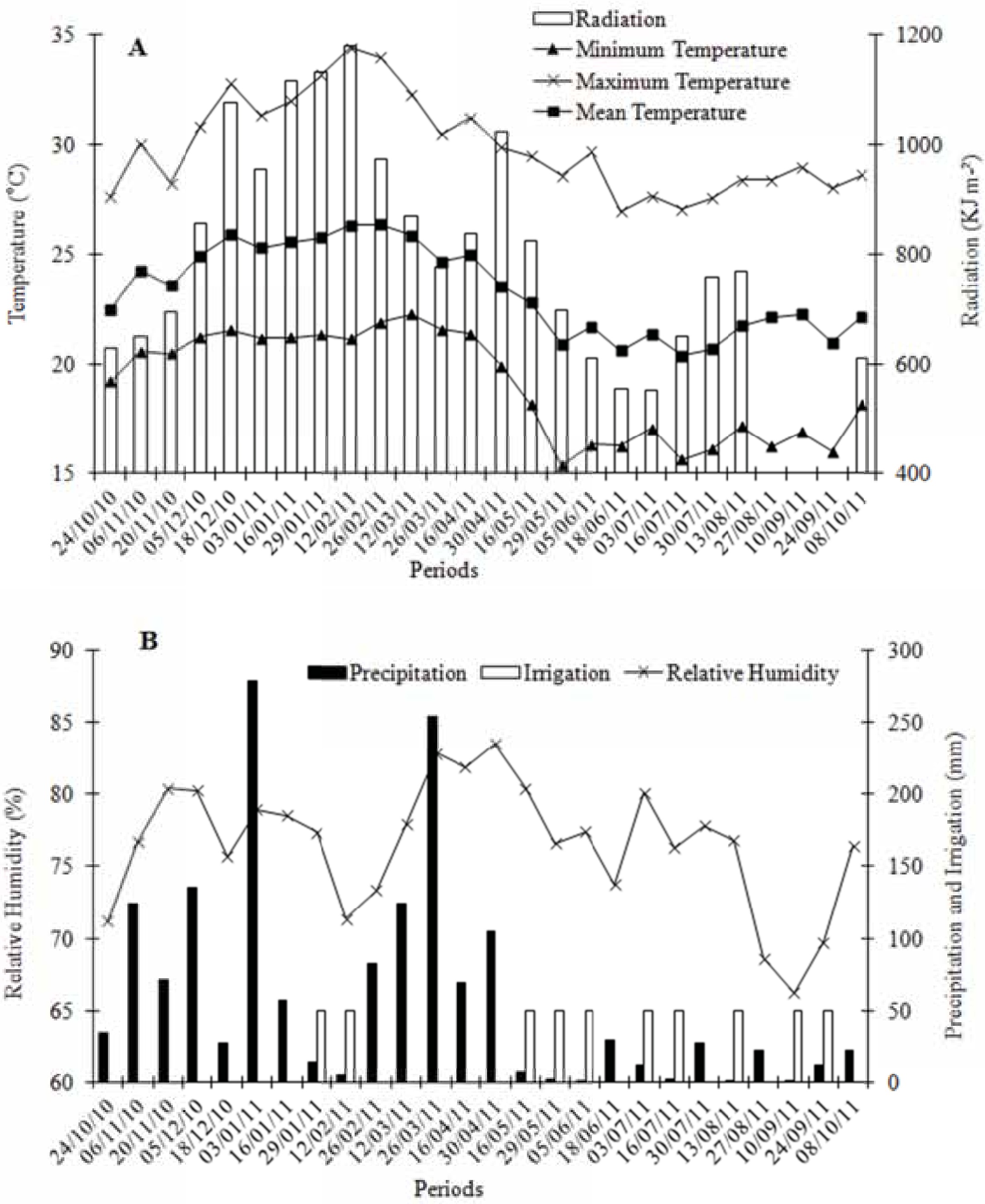
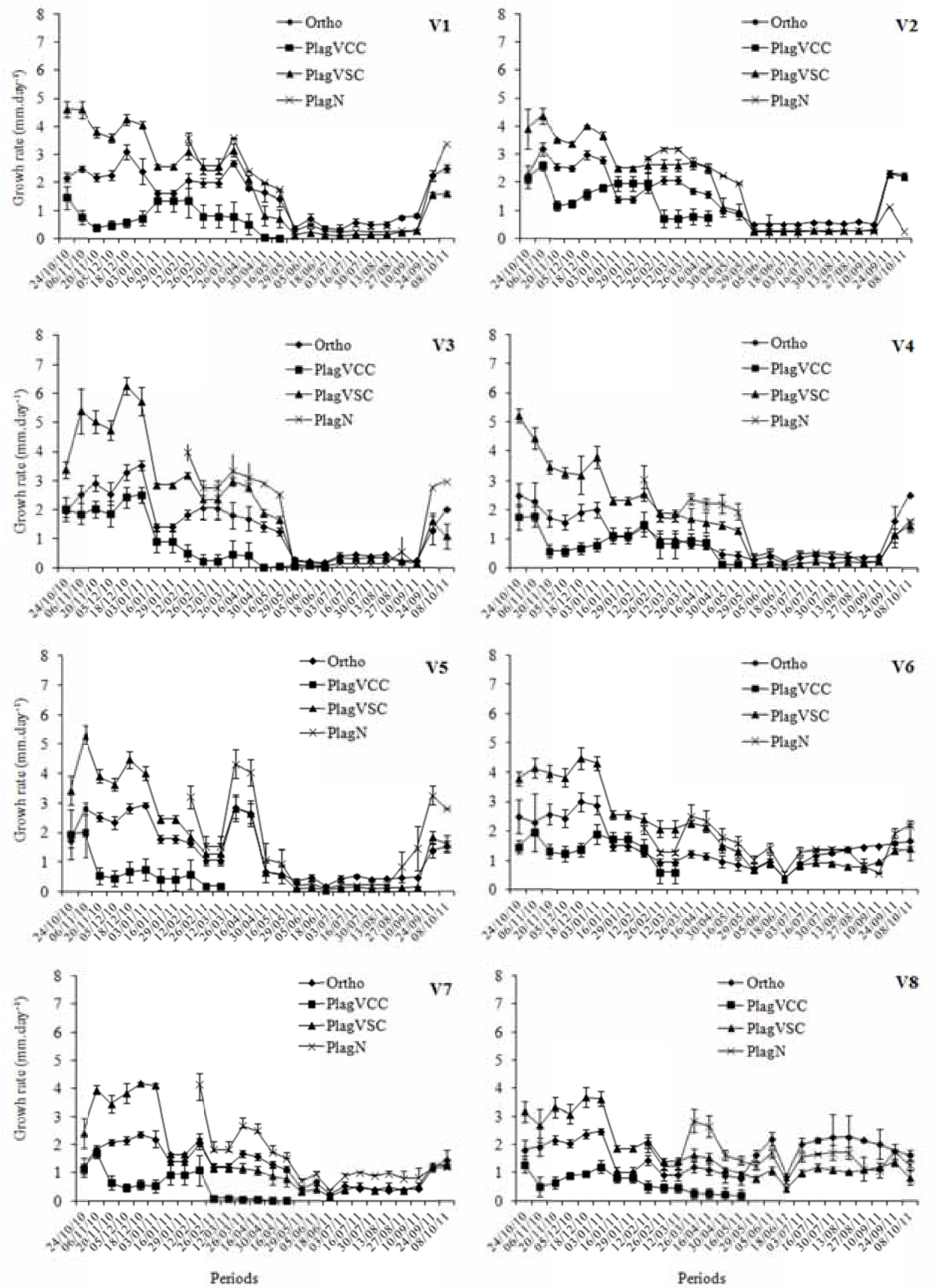


Figure 1. Averages for minimum, maximum, and mean temperature, radiation (A), precipitation, irrigation, and relative humidity (B) measured during the experiment. Nova Venécia/ES, 2010/2011. Note: It was not possible to obtain radiation data from August 13 to September 24


V1
V2
V3
V4
V5
V6
V7
V8
Ortho
PlagVCC
PlagVSC
PlagN
Growth rate (mm.day-1)
Periods

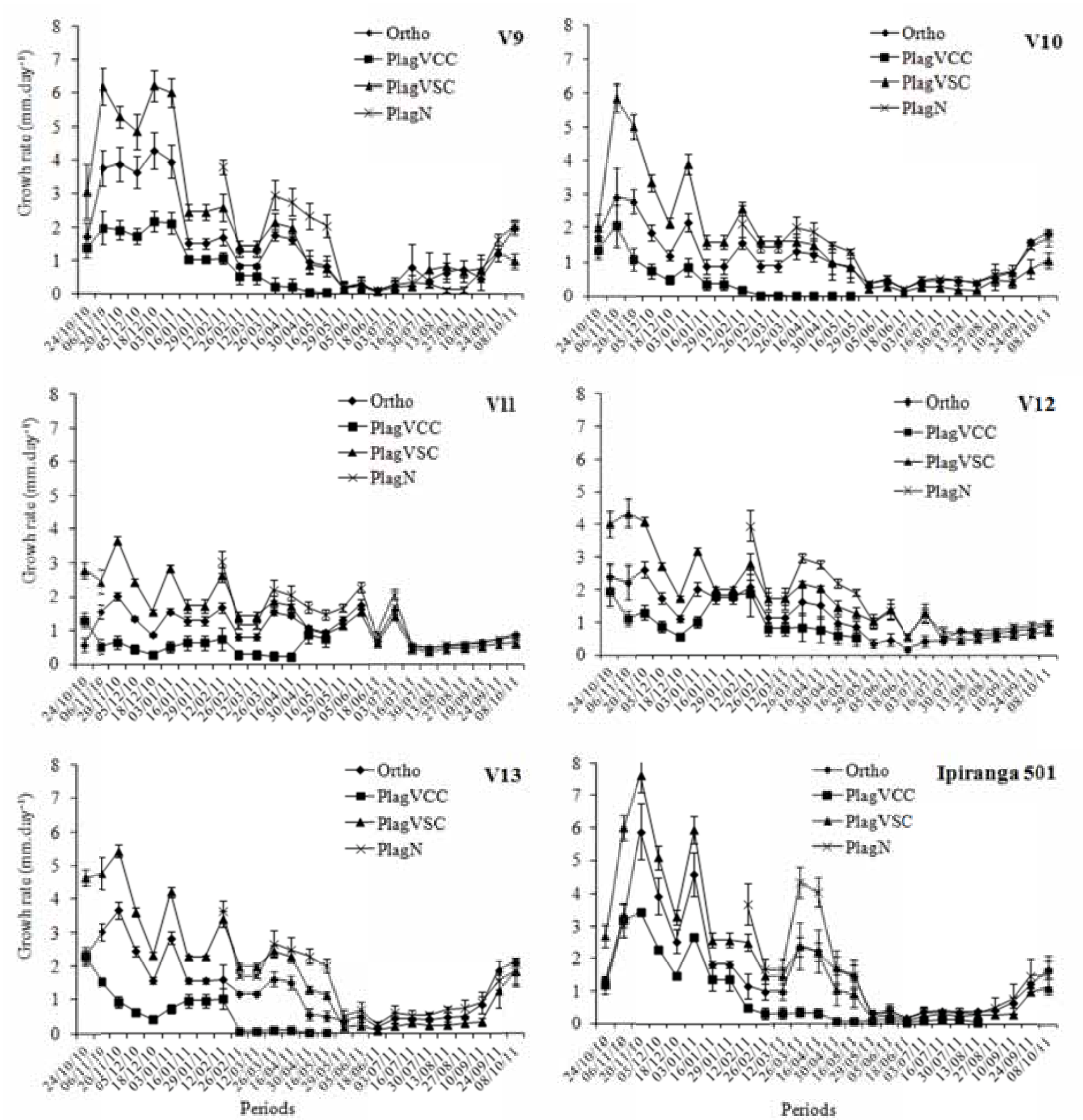


Figure 2. Vegetative growth rate (mm day^{-1}) of groups of orthotropic and plagiotropic branches along one-year experiment in 14 genotypes of *C. canephora*. Each point represents the mean + SE (n = 05)

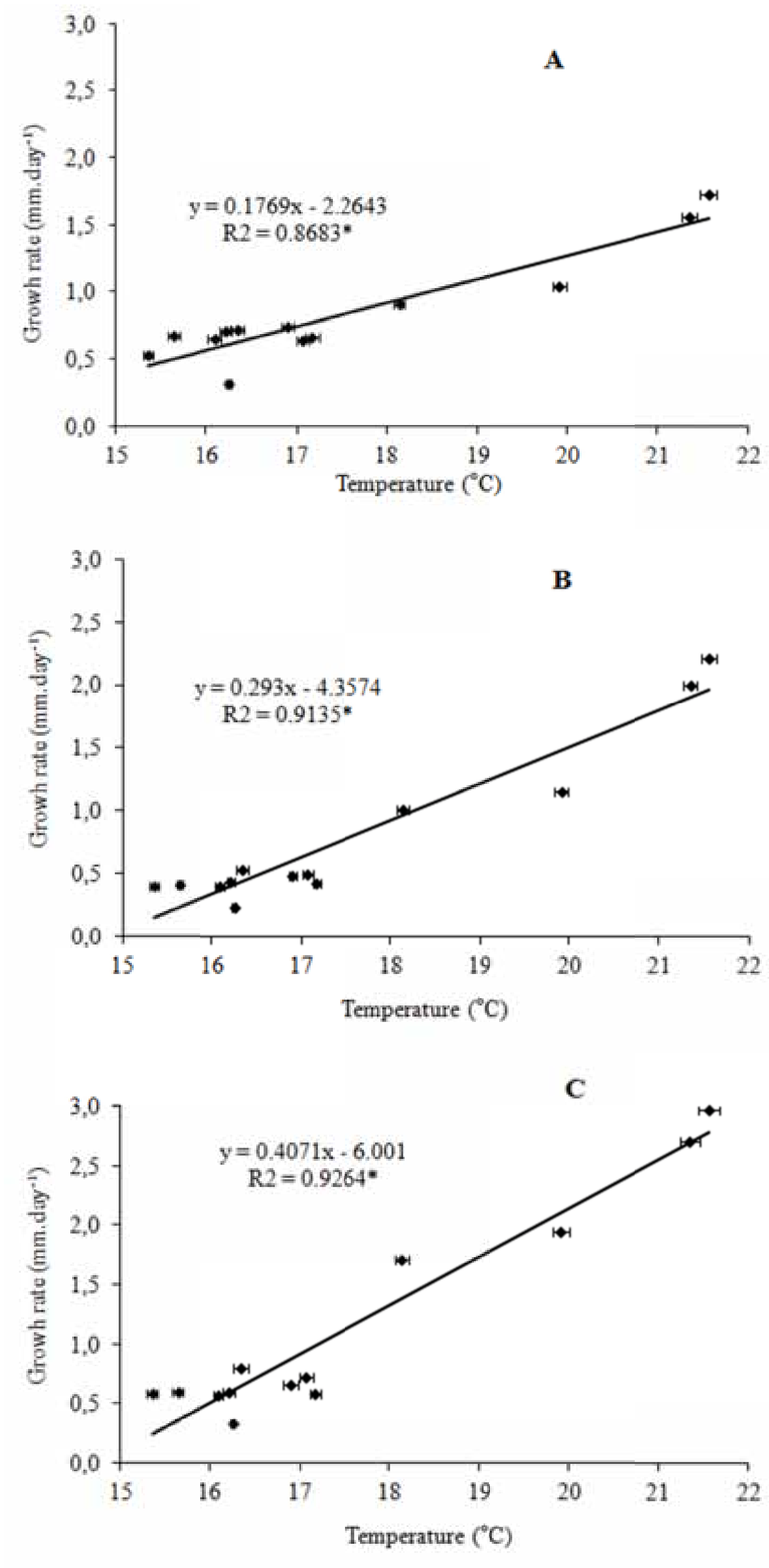


Figure 3. Association between the average minimum temperature (measured between 26/03/2011 and 10/09/2011 - autumn/winter) and the vegetative growth rate of orthotropic branches (A), old plagiotropic branches without berries (B), and new plagiotropic branches (C)

4. Conclusion

Under our experimental conditions, the growth rate of the orthotropic and plagiotropic branches of *C. canephora* differed according to the genotype and underwent seasonal variation throughout the year, which was mainly influenced by changes in the air temperature. Under minimum air temperatures below 17.2°C, the growth rate of *C. canephora* branches was sharply reduced for most of the genotypes studied. Yet, strong differences were found amongst genotypes, with V8 being the one that maintained an appreciable growth rate of most of branch groups along almost the entire year. Over the course of months, plagiotropic branches, mainly those with berries, presented less vegetative growth when compared to the younger branches.

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Resistance to the Pink Stem Borer in Twenty Exotic Maize Populations Under Natural and Artificial Infestation Conditions

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Abstract

Twenty exotic maize populations with different genetic background representing different geographical zones were obtained from Maize Gene Bank of Maize Res. Dept., Field Crops Res. Inst. (FCRI), Agric. Res. Center (ARC), Egypt. They were evaluated in 2011 to determine their level of resistance to the pink stem borer *Sesamia cretica* under natural infestation at Nubaria, Gemmeiza and Sakha Agric. Res. Stn. and under artificial infestation at Giza Agric. Res. Stn. A Randomized Complete Block Design was used. Evaluation trials under natural infestation were planted in the early summer (April) while under artificial infestation planting took place in the normal growing season (May). Three resistance expressing traits, i.e. percentage of infested plants (IP), percentage of plants with dead hearts (DH) and intensity of damage (ID) were used to evaluate the level of resistance in the twenty populations. Five-classes rating scale was developed to evaluate the intensity of damage caused by larvae of *S. cretica*. Results showed that, populations Tamps. 23 and Antigua have relatively good level of resistance to infestation by larvae of *S. cretica*. The two populations could be integrated into maize breeding programs which aim at developing hybrids resistant to *S. cretica*. Highly significant correlation coefficients were found among pairs of the three resistance expressing traits under artificial infestation. Correlation between results under natural and artificial infestation for the three studied traits showed low to moderate correlation which indicated that, for precise results on the level of resistance to *S. cretica*, evaluation should be made under artificial infestation conditions. In addition, exotic germplasm should be considered as a source for resistance genes in breeding programs for insect-pest resistance.

Keywords: pink stem borer, Sesamia cretica, natural and artificial infestation, correlation coefficients, exotic germplasm

1. Introduction

Maize is considered as one of the most important cereal crops in Egypt. It can play a major role in narrowing the present food gap via expansion in cultivation of maize hybrids with high yield potentiality and resistant to major diseases and insect pests affecting maize production.

In Egypt, maize plants are usually attacked by several injurious insect pests. The pink stem borer, *Sesamia cretica* Led. and the European corn borer, *Ostrinia nubilalis* (Hub.) are considered the major insects affecting maize productivity since they can cause great yield losses. James (2003) stated that 9% of the world maize crop is lost annually due to damage caused by insect pests. Oloyede et al. (2011) found that grain yield losses due to *S. calamistis* infestation ranged from 25-30%.

The pink stem borer *S. cretica* is considered the most damaging corn borer in Egypt since it attacks young maize plants shortly after emergence, devours the whorl leaves and may kill the growing point causing complete death of small maize plants and consequently reducing number of plants at harvest causing drastic yield losses (Semeada, 1985; EL-Naggar, 1991; Soliman, 1994). This insect is also capable of damaging older plants through excavating tunnels into the stems, ears and/ or cobs. Yield reduction due to *S. cretica* depends on many factors including the stage at which the plant is infested, the insect population available for infestation in addition to the environmental conditions during the growing season.

Several investigations were carried out to evaluate maize cultivars for their resistance to the pink stem borer, most of them were conducted in Egypt either under natural infestation (Simeada, 1985; EL-Sherif et al., 1986; Tantawy et al., 1989; Al-Naggar et al., 2000b; Soliman et al., 2001, Malver et al., 2007; Hemida et al., 2008) or under artificial infestation (EL-Sherif & Mostafa, 1987; Al-Naggar et al., 2000b; Soliman et al., 2001; Sekhara et al., 2008; Oloyede, 2011; El-Rawy & Abdalla, 2011; Santosh et al., 2012; Ismail, 2012) or under both natural and artificial infestation (Soliman, 1997; Soliman et al., 2001; Galal et al., 2002; Mosa & Amer, 2004; Mourad & El-Rawy, 2012). However, most researchers working on host plant resistance to corn borers other than *S. cretica* are convinced that artificial infestation is superior and more efficient than natural infestation for accurate differentiation among maize genotypes for their resistance to corn borers (Guthrie, 1982; Mihm, 1983a). However, in case where there is a need to screen a large number of maize genotypes and the environmental conditions allow for uniform and heavy infestation with larvae of *S.cretica*, evaluation under natural infestation would allow for obtaining primary evaluation of these genotypes especially if evaluation can be achieved over a wide range of environments. Later, artificial infestation can be performed but will be, in this case, with a smaller number of genotypes for results confirmation.

The objectives of the present investigation were to: 1- determine the level of resistance to *S. cretica* in 20 exotic maize populations under natural and artificial infestation conditions, 2-assess the consistency of resistance results obtained from both methods of evaluation and 3- to decide if any of these populations can be integrated into the national breeding program that aims at developing maize hybrids resistant to the pink stem borer.

2. Materials and Methods

Twenty exotic maize populations were chosen for the purpose of this investigation. They were obtained from the Gene Bank of Maize Research Department, Field Crops Research Institute (FCRI) Agriculture Research Center (ARC), Egypt. These populations have different genetic backgrounds representing different geographical zones (Table 4).

Planting of these populations under natural infestation of *S. cretica* was carried out at Nubaria (29°30' longitude, and 30°54' latitude), Gemmeiza (31°00' longitude and 30°83' latitude) and Sakha (30°98' longitude and 31°11' latitude), Agric. Res. Stn. of ARC on April 6th, 19th and 21st, 2011, respectively. Day temperatures during summer growing season are from 27-33°C at Nubaria and Sakha and from 29-35°C at Gemmeiza location. High humidity is normally prevailing during July-August. These planting dates were chosen so that the plants reach the growth stage most preferred by this insect and the moth emergence from hibernating larvae reached its maximum to assure maximum natural infestation (Ahmed & Kira, 1960). These populations were also evaluated under artificial infestation of at Giza Agric. Res. Stn. of ARC. Sowing date for artificial infestation was on May 30th, 2011. This planting date was chosen to coincide with the time of minimum natural infestation and so, minimizing intervening of natural and artificial infestation which can affect obtained results. Artificial infestation was performed by newly hatched larvae artificially reared in the Corn Borers Lab of Maize Res. Dept., FCRI, ARC using the Bazoka equipment as a mechanical dispenser according to Mihm (1983 a). Infestation was applied two times to ten plants in each row at 20-25 days after planting at the early whorl stage. Splitting of infestation was to assure occurrence of infestation. Each plant received 8-10 larvae in each application.

Randomized Complete Block Design with four replicates was used in all testing locations. Plot size under natural infestation was 4 rows of 6 m length, 60 cm width, with hills spaced at 25 cm, while for artificial infestation, the experimental plot was one row 4 m length, 70 cm width and 25 cm hill spacing. Technical recommendations for maize growing were applied except pest control which was entirely avoided. Required field data were recorded at all testing locations 35 days after planting in case of evaluation under natural infestation and after 15 days from infestation of plants when evaluation was carried out under artificial infestation.

Data were recorded under natural artificial infestation on the following traits using five-classes rating scale which can accurately differentiate among tested genotypes.

2.1 Percentage of Infested Plants

$$\frac{No. of\ infested\ plants\ plot^{-1} \times 100}{Total\ no. of\ plants\ plot^{-1}}$$

Genotypes were classified according to their mean percentage of infested plants into: Resistant, R (less than 30%). Moderately Resistant, MR (from 30% to less than 40%), Intermediate, I (from 40% to less than 50%), Susceptible, S (from 50% to less than 60%), and Highly Susceptible, HS (more than 60%).

2.2 Percentage of Plants with Dead Hearts

$$\frac{No. of\ plants\ with\ dead\ hearts\ plot^{-1} \times 100}{Total\ no. of\ plants\ plot^{-1}}$$

Genotypes were classified according to their mean percentage of plants with dead hearts into: Resistant, R (less than 8%). Moderately Resistant, MR (from 8% to less than 12%), Intermediate, I (from 12% to less than 16%), Susceptible, S (from 16% to less than 20%), and Highly Susceptible, HS (more than 20%).

2.3 Intensity of Damage

Five-classes rating scale was developed to evaluate the intensity of damage caused by attack of *S. cretica* larvae. The description of this scale is as follows:

Class 1: No visible injury on plants (no symptoms).

Class 2: Less than 25% of plants plot^{-1} is with holes less than 1 mm in diameter across partially or fully unfolded whorl leaves.

Class 3: Several folded and unfolded whorl leaves with relatively larger round and/or elongated holes accompanied with small yellowish green pillets of frass aggregated in the whorl.

Class 4: Plants with relatively larger round and/or elongated irregular holes, evident distortion of the leaves (most leaves have long holes), withering of whorl and accumulation of comparatively large sized pellets of frass in the whorl or on the ground around the stem.

Class 5: Plants with dead hearts.

The intensity of damage (ID) value for each plot was calculated as follows**:**

$$ID = \frac{ID1 + ID2 + ... + IDn}{N}$$

Where, N is the number of inspected plants.

Genotypes were classified according to their ID into: Resistant, R (less than 1.5), Moderately Resistant, MR (from 1.5 to less than 1.9), Intermediate, I (from 1.9 to less than 2.3), Susceptible, S (from 2.3 to less than 2.7), and Highly Susceptible, HS (more than 2.7).

Data that were collected in percentage were subjected to arcsine transformation for the purpose of statistical analysis. However, such data were presented hereafter in original percentages. Due to heterogeneity of error variances at the three testing locations, combined analysis was performed. Correlation coefficients among the three resistance expressing traits were also computed. All statistical analyses were performed according to Steel and Torrie (1980).

3. Results and Discussion

3.1 Analysis of Variance

Combined analyses of variance of the twenty maize populations for the three characters expressing maize plant resistance to *Sesamia cretica* (percentage of infested plants, percentage of plants with dead hearts, and intensity of plant damage) under natural infestation are presented in Table 1, while analysis of variance for the same characters under artificial infestation is presented in Table 2. Locations mean squares were highly significant for all studied traits (Table 1) indicating differences in the climatic conditions among the three testing locations at time of infestation which resulted in performance differences among the tested populations. Either significant or highly significant differences were detected among the 20 populations for the three resistance expressing traits under natural and artificial infestation (Tables 1 and 2). This indicated the presence of real differences in the genetic constitution of these populations concerning their level of resistance to infestation by the pink stem borer. Location×entry mean squares (Table 1) were highly significant for percentage of infested plants and plants with dead hearts which showed that the 20 populations behaved differently from one location to another and also indicated the importance of multilocation testing.

Table 1. ANOVA analysis to evaluate significant differences among the twenty maize populations evaluated under natural infestation across three locations in 2011

S.O.V.	d.f.	Infested Plants	Dead Hearts	Intensity of Damage
Locations (Loc.)	2	6491.9**	6685.2**	46.63**
Rep's (Loc)	9	52.5**	19.4*	0.42**
Populations (Pop)	19	110.8**	29.3**	0.28*
Loc × Pop	38	39.9**	32.1**	0.21
Error	171	16.8	9.9	0.14

Table 2. ANOVA analysis to evaluate significant differences among the twenty maize populations evaluated under artificial infestation in 2011

S.O.V.	d.f.	Infested Plants	Dead Hearts	Intensity of Damage
Replications	2	232.53**	1.94	0.116*
Populations	19	470.36**	83.48**	2.240**
Error	38	15.81	1.63	0.027

3.2 Mean Performance of Populations

Mean performances and insect reaction scores of the twenty populations under natural (combined) and artificial infestation are presented in Tables 3 and 4, respectively. Results for level of resistance under natural infestation, at separate locations, are presented in Table 5. Results varied considerably from one location to another where Sakha location had severe infestation while Nubaria location had the lowest and could not discriminate precisely among tested populations.

Table 3. Mean performance for percentage of infested plants, percentage of plants with dead hearts and intensity of damage under natural and artificial infestations of S. cretica, in 2011

Entry No.	Population	Infested plants (%)		Plant with dead hearts (%)		Intensity of damage	
		Natural	Artificial	Natural	Artificial	Natural	Artificial
1	V.55	30.8	43.9	19.8	26.4	2.03	2.62
2	Yellow Kenya	31.9	42.2	17.9	23.4	2.18	2.48
3	Iowatigua- Tep 953	29.4	50.2	17.6	27.7	2.05	3.27
4	Carotigua-Tep 955	33.2	49.1	20.7	26.0	2.17	2.93
5	Tamps-8	29.5	45.0	15.9	21.7	2.05	2.39
6	Tamps-23	28.6	37.2	15.0	15.0	1.89	2.10
7	Thick Rind	35.7	48.3	18.1	24.2	2.31	3.05
8	Antigua	28.7	38.9	17.3	15.0	2.05	2.24
9	Westigua	34.8	61.5	17.4	24.0	2.20	3.74
10	Pool-29	35.3	64.5	18.6	27.9	2.37	4.07
11	Pool-30	40.9	78.0	20.6	31.1	2.44	4.82
12	Pool-33	33.9	54.2	18.3	26.0	2.37	3.49
13	Pool-34	33.7	57.6	18.7	27.1	2.30	3.74
14	Mexico-207	31.9	55.9	16.9	24.8	2.04	3.57
15	Sonora GP.03	33.2	61.9	19.4	26.1	2.27	3.44
16	Guat-104	30.2	70.0	16.5	29.0	2.07	4.38
17	Managua-7432	33.9	64.2	18.4	27.3	2.18	3.81
18	Tep.5	33.4	78.0	20.3	38.4	2.20	4.77
19	Tuxpeno	31.3	70.3	16.2	32.8	2.11	4.65
20	Lancaster	28.4	69.3	16.6	30.0	1.91	4.50
Mean		32.4	57.0	18.0	26.2	2.2	3.5
C.V.		12.6	7.0	17.4	4.9	17.6	4.7
LSD(0.05)		3.29	6.57	2.53	2.11	0.31	0.27

Table 4. *Pink stem borer* resistances scores of the tested populations under natural (Nat.) and artificial (Art.) infestation across locations, in 2011

Entry No.	Pedigree	Origin	I.P.*		D.H.		I.D.	
			Nat	Art	Nat	Art	Nat	Art
1	V.55	CIMMY**	MR	I	S	HS	I	HS
2	Yellow Kenya	Kenya	MR	I	S	HS	I	HS
3	Iowatigua- Tep 953	CIMMYT	R	S	S	HS	I	HS
4	Carotigua-Tep 955	CIMMYT	MR	I	HS	HS	I	HS
5	Tamps-8	CIMMYT	R	I	I	HS	I	HS
6	Tamps-23	CIMMYT	R	MR	I	I	MR	I
7	Thick Rind	USA	MR	I	S	HS	I	HS
8	Antigua	CIMMYT	R	MR	S	I	I	I
9	Westigua	CIMMYT	MR	S	S	HS	S	HS
10	Pool-29	CIMMYT	MR	S	S	HS	S	HS
11	Pool-30	CIMMYT	I	HS	HS	HS	S	HS
12	Pool-33	CIMMYT	MR	S	S	HS	S	HS
13	Pool-34	CIMMYT	MR	S	S	HS	I	HS
14	Mexico-207	CIMMYT	MR	S	S	HS	I	HS
15	Sonora GP.03	CIMMYT	MR	HS	S	HS	I	HS
16	Guat-104	CIMMYT	MR	HS	S	HS	I	HS
17	Managua-7432	CIMMYT	MR	HS	S	HS	I	HS
18	Tep.5	CIMMYT	MR	HS	HS	HS	I	HS
19	Tuxpeno	CIMMYT	MR	HS	S	HS	I	HS
20	Lancaster	USA	R	HS	S	HS	I	HS

*. I.P., D.H., and I.D: Infested plants (%), Dead hearts (%) and intensity of damage, respectively.

**. CIMMYT: International Center for Maize and Wheat Improvement, located in El-Batan, Mixico. Genetic stocks introduced from CIMMYT represent mainly Latin American germplasm.

Table 5. Mean performance for resistance expressing traits at Nubaria, Gemmeza and Sakha research stations under natural infestation of *S. cretica* in 2011

Ent no.	population	Nubaria						Gemmeiza						Sakha					
		I.P.%		D.H.%		I.D.		I.P.%		D.H.%		I.D.		I.P.%		D.H.%		I.D.	
		score	scale	score	scale	score	scale	score	scale	score	scale	score	scale	score	scale	score	scale	score	Scale
1	V.55	27.1	R	14.4	I	1.6	MR	26.9	R	17.0	S	1.8	MR	38.4	MR	28.1	HS	2.8	HS
2	Yellow Kenya	27.6	R	12.3	I	1.5	MR	26.0	R	14.4	I	1.8	MR	42.2	MR	26.8	HS	3.2	HS
3	Iowatigua Tep 953	20.0	R	8.5	MR	1.5	MR	28.7	R	15.7	I	1.9	I	39.7	I	28.5	HS	2.8	HS
4	Carotigua Tep 955	27.3	R	17.6	I	1.8	MR	29.2	R	16.6	S	1.8	MR	43.1	MR	27.8	HS	2.9	HS
5	Tamps-8	25.3	R	9.8	MR	1.5	MR	26.4	R	14.9	I	1.7	MR	36.9	MR	23.7	HS	2.8	HS
6	Tamps-23	26.9	R	9.1	MR	1.4	MR	25.3	R	16.0	I	1.7	MR	33.6	MR	20.8	HS	2.4	HS
7	Thick Rind	28.6	R	9.7	MR	1.6	MR	34.1	MR	20.2	S	2.3	S	44.4	S	24.5	HS	3.0	HS
8	Antigua	19.5	R	9.8	MR	1.4	MR	30.9	MR	20.3	S	2.1	I	35.7	I	22.7	HS	2.6	S
9	Westigua	25.4	R	6.6	R	1.4	MR	34.6	MR	16.9	S	2.1	I	44.5	I	28.6	HS	3.0	HS
10	Pool-29	25.2	R	10.0	MR	1.5	MR	32.9	MR	16.0	I	2.4	S	47.8	S	29.5	HS	3.2	HS
11	Pool 30	28.0	R	9.2	MR	1.6	MR	37.9	MR	16.7	S	2.2	I	56.8	I	36.1	HS	3.5	HS
12	Pool 33	26.0	R	7.2	R	1.5	MR	30.8	MR	19.1	S	2.0	I	45.0	I	28.7	HS	3.7	HS
13	Pool 34	24.4	R	9.8	MR	1.5	MR	32.5	MR	18.9	S	2.3	S	44.0	S	27.5	HS	3.0	HS
14	Mexico-207	23.5	R	7.5	R	1.5	MR	30.1	MR	15.1	I	1.8	MR	42.0	MR	28.2	HS	2.9	HS
15	Sonora GP 03	28.4	R	10.6	MR	1.7	MR	28.0	R	18.5	S	1.8	MR	43.2	MR	29.0	HS	3.3	HS
16	Guate-104	20.1	R	7.1	R	1.4	MR	28.3	R	12.0	I	1.6	MR	41.5	MR	30.4	HS	3.1	HS
17	Managua-7432	23.1	R	7.0	R	1.4	MR	31.8	MR	16.7	S	1.8	MR	46.8	MR	31.4	HS	3.4	HS
18	Tep-5	24.8	R	11.2	MR	1.6	MR	33.1	MR	19.6	S	2.0	I	42.3	I	30.2	HS	3.0	HS
19	Tuxpeno	25.1	R	8.3	MR	1.6	MR	23.1	R	14.5	I	1.6	MR	45.6	MR	25.7	HS	3.1	HS
20	Lancaster	23.4	R	6.6	R	1.5	MR	26.0	R	17.5	S	1.8	MR	35.3	MR	25.6	HS	2.4	S
	Mean	25.0		9.5		1.6		30.0		16.8		1.9		42.8		27.7		1.9	
	Scale	R		MR		MR		MR		S		I		I		HS		I	
	C.V.	14.2		22.4		14.9		11.3		17.2		15.7		12.0		15.0		18.0	

3.2.1 Infested Plants (%)

Results in Tables 3 and 4 showed that, under natural infestation, only five populations, i.e. Iowatigua, Tamps.8, Tamps.23, Antigua and Lancaster were significantly resistant, while all the other populations were moderately resistant except Pool 30 which was intermediate. However, under artificial infestation only two populations, i.e.Tamps.23 and Antigua were moderately resistant and five entries, i.e. V. 55, Yellow Kenya, Carotigua, Tamps.8, and Thick Rind were intermediate. No strong consistency was found for results of resistance under natural and artificial for this trait, indicating that selection of resistant genotypes should mainly depends on artificial infestation.

3.2.2 Plants with Dead Hearts (%)

Results for resistance under natural infestation showed that populations Tamps.8 and Tamps.23 were intermediate while the other 18 populations were either susceptible or highly susceptible. Under artificial infestation, populations Tamps. 23 and Antigua were intermediate while the other populations were highly susceptible (Tables 3 and 4). Results of this trait under artificial infestation were, to some extent, similar to those under natural infestation.

3.2.3 Intensity of Damage

Results on intensity of damage (Tables 3 and 4) revealed that, under natural infestation only one population (Tamps. 23) was moderately resistant, 14 populations were of intermediate resistance , and five populations were susceptible. Results under artificial infestation showed that only two populations (Tamps. 23 and Antigua) were of intermediate resistance while the remaining populations were highly susceptible. Results showed also that, scores for the three resistance traits were much higher under artificial infestation as compared to those under natural infestation. This indicated that natural infestation in 2011, in general, was not severe enough to accurately assess the intensity of damage caused by larvae of S. cretica except at Sakha location, while artificial infestation had enforced good infestation pressure on the tested populations and was successful in discovering differences among them concerning resistance to infestation by larvae of *S. cretica*.

The top performing populations that behaved as resistant, moderately resistant or intermediate under both natural and artificial infestation were considered. Based on this criterion, populations Tamps. 23 and Antigua were considered of usefulness and could be integrated in the national maize breeding program for developing maize hybrids with resistance to infestation by larvae of *S. cretica* as well as high yield potentiality. Results revealed also that, out of the twenty exotic populations only two populations (10%) had relatively good level of resistance to *S.cretica*.The role of exotic germplasm as a source for resistance to *Sesamia spp*.was investigated by many researchers. Burton et al. (1999) screened 121 exotic maize inbred lines representing seven germplasm groups and they found that only 7 inbred lines (6%) were considered resistant to *S. nonagrioidis*. Santosh et al. (2012) evaluated 48 exotic inbred lines for resistance to *S. inferences* and found that only 8 inbred lines (16%) were resistant. Mourad and El-Rawy (2012) evaluated 13 exotic sorghum lines for resistance to *S. cretica* and only one line (8%) was found resistant. Results of this investigation indicated that natural infestation with *S. cretica* was not able to discriminate precisely between resistant and susceptible populations for the three resistance expressing traits. This conclusion was reached also by Soliman (1997) and Soliman et al. (2001). They indicated that maize breeders should depend only on artificial infestation in order to get precise results about the level of resistance to *S. cretica* in the genotypes under study. However, contradictory results were obtained by Tantawi et al. (1989) where they found that artificial infestation was successful in differentiating between resistant and susceptible hybrids. Also, Galal et al. (2002) indicated that natural infestation was suitable for studying the genetic behavior of resistance to the pink stem borer. Investigations of the last two researchers may be conducted in some years where environmental conditions allowed for high and uniform infestation levels.

Table 6. Correlation coefficients among pairs of the three resistance expressing traits under artificial and natural infestation, in 2011

Traits	I.P.		D.H.		I.D.	
	Nat	Art	Nat	Art	Nat	Art
Infested plants (%), IP	--	--	0.938**	0.768**	0.730**	0.919**
Dead heart plants (%), DH	--	--	--	--	0.572**	0.827**
Intensity of damage, ID	--	--	--	--	--	--

Table 7. Correlation coefficients between results under natural and artificial infestation for the three resistance expressing traits, in 2011

TRAIT	I.P. (natural)	D.H. (natural)	I.D. (natural)
I.P.+ (artificial)	0.475**	….	….
D.H.+ (artificial)	….	0.331**	….
I.D.+ (artificial)	….	….	0.262*

+ I.P., D.H., and I.D.: refer to infested plants (%), plants with dead hearts (%) and Intensity of damage, respectively.

3.3 Correlation among Resistance Traits

Simple correlation coefficients among pairs of the three resistance expressing traits, i.e. percentage of infested plants (IP), percentage of plants with dead hearts (DH) and intensity of damage (ID) are presented in Table 6. Results showed that, under natural infestation, correlation coefficients between IP and DH, IP and ID, DH and ID were 0.94, 0.73 and 0.57, respectively, while under artificial infestation, the correlation coefficients were (0.77, 0.92 and 0.83), respectively. Results confirmed the presence of highly significant positive correlation among the three traits and that; these positive correlations were higher, except for percentage of infested plants, under artificial infestation as compared to those under natural infestation conditions. These results indicated also that, only one or two of the three resistance expressing traits can be used in S. cretica resistance investigations. Using percentage of plants with dead hearts and intensity of plant damage should be enough in evaluation experiments for resistance to *S. cretica.* This is due to their effect on plant growth and number of plants at harvest and consequently obtained yield. Similar results were obtained by Odiyi (2007) who studied the effect of infestation by *S. calamistis* on grain yield and found moderate to high correlations among most pairs of resistance expressing traits.

Correlation coefficients between results under natural and artificial infestation for each of the three resistance expressing traits are presented in Table 7. Significant or highly significant positive correlation coefficients were obtained, but the correlation was not strong enough since the correlation values were low to moderate. This emphasizes the need for artificial infestation in order to get precise results for the level of resistance in any tested genotypes. Similar results were reported by Soliman (1997) who found low correlation between results under natural and artificial infestation by *S. cretica.*

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Nutritional Efficiency of Phosphorus in Lettuce

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Abstract

This work aimed to evaluate the efficiency of phosphorus absorption, translocation, and utilization in lettuce. A greenhouse experiment was arranged in a completely randomized design in a 5x2 factorial scheme to test five phosphorus doses (0, 100, 200, 300, and 500 mg dm^{-3}) and two lettuce varieties (Lisa and Americana). The soil was incubated with limestone for pH correction. Following this procedure, a phosphate fertilization was carried and the lettuce seedlings were transplanted. Fertilization with nitrogen and potassium were applied as cover. Forty days after seedling transplantation, the plants were separated into root and aerial part to determine the following characteristics: aerial part dry matter (APDM); root dry matter (RDM); phosphorus content in the aerial part (PCAP); phosphorus content in the root (PCR); phosphorus accumulation in the aerial part (PAAP); phosphorus accumulation in the roots (PAR); phosphorus absorption efficiency (PAE); phosphorus translocation efficiency (PTE); phosphorus use efficiency in the aerial part (PUEAP) and root-aerial part ratio (R/AP). The results obtained allowed to conclude that RDM and R/AP had a significant interaction between the varieties and the P doses, with Lisa presenting the highest values of these characteristics at the lowest doses evaluated. The characteristics PCAP, PAAP and PAE presented a significant difference between the varieties, with the Americana presenting the highest values. The characteristics PCAP, PAAP, EAP, APDM and PUEAP presented a significant difference between the P doses, with the highest values found at the dose of 500 mg dm^{-3}, except for PUEAP, whose dose was 0 mg dm^{-3}.

Keywords: mineral nutrition, phosphorus use efficiency, phosphorus acquisition

1. Introduction

Lettuce is a leafy vegetable known and cultivated worldwide. It is adapted to mild temperature and adequate to fall/ winter cultivation. It is currently one of the most popular vegetables, being cultivated throughout Brazil (Lima, 2005). In the summer, its reduced offer/demand ratio generally provides higher prices. Its consumption is mostly "in natura" (Filgueira, 2003).

Since lettuce is originated from temperate climate, its adaptation to high temperature regions has generated obstacles to its growth and development, preventing the culture from expressing its total genetic potential. Protected cultivations and the use of cultivars adapted to different conditions of cultivation allow this culture to be available in the market throughout the year (Filgueira, 2003). Lettuce cultivation is of easy management, presenting a short cycle that ensures a rapid return of the capital invested. Among the leafy vegetables, lettuce is the most planted and consumed by the Brazilian population. The state of São Paulo is Brazil's main vegetable producer and has the largest consumer market (Camargo Filho & Camargo, 2008).

Lettuce nutrition is of the utmost importance to guarantee quality production. Thus, fertilization management must be known and planned for this purpose. Besides, nutrition with phosphorus presents peculiarities such as differences in absorption between genotypes (Cock, Amaral Júnior, Smith, & Monnerat, 2002) and their capacity of adsorption in tropical soils, decreasing its availability. This decreases the phosphorus contents available for the plant in the soil's solution. Without considering type of lettuce, the use of 200 to 400 kg ha^{-1} of P_2O_5 has been recommended for phosphate fertilization, according to type of soil and availability of this nutrient in the soil (Filgueira, 2003). Lettuce can be considered quite phosphorus-demanding, especially at the final phase of its cycle, since this macronutrient occurs in various compounds and metabolic reactions (Marschner,1995).

Phosphorus deficiency reduces plant growth, causing head malformation, opaque-green coloration of the old leaves, possibly presenting bronze- red or purple coloration (Katayama, 1993). Absence of P causes a significant reduction in fresh matter production of the plant's aerial part and roots, significant reduction in the plant's diameter and of its content in the leaves, evidencing lettuce's great demand for this nutrient (Lana, ZanãoJúnior, Luz, & Silva, 2004).

Thus, the objective of this work was to evaluate the efficiency of phosphorus absorption, translocation, and use in two lettuce varieties and at different doses of phosphorus.

2. Material and Methods

This work was conducted in $3dm^3$ vases, from May to June 2012, under greenhouse conditions, at the Agrarian Sciences Center of the Universidade Federal do Espírito Santo, in Alegre-ES, with geographic coordinates of latitude 20º45' South, longitude 41º48' West and altitude 147 m. The soil used was collected from Red-Yellow Latosol, from which a sample was taken and sent to the laboratory for determination of the chemical attributes (Table 1) and texture (Table 2), according to the methodology described by Brasilian Company Agropecuaria-Embrapa (2009).

Table 1. Chemical attributes of the soil used in the experiment

pH	P	K	Na	Ca	Mg	Al	H+Al	t	T	SB	V	m
H_2O	-----mg dm^{-3}-----		--------------------$cmol_cdm^{-3}$--------------------								------%-----	
5.5	15.03	32.0	2.0	2.37	0.32	0.2	5.78	2.98	8.56	2.78	32.53	6.7

Where, t = Effective cationic exchange capacity; T = Cationic exchange capacity at pH 7 (CTC); SB = Sum of exchangeable bases; V = Saturation Index in bases and m =Saturation Index in aluminum.

Table 2. Texture of the soil used in the experiment

Sand	Silt	Clay	Texturalclass
----------%---------			
46	9	45	(clayey)

Due to its high clay content (45%-Table 2), what would hinder root development, the soil used in the experiment was mixed with washed sand (3:1 (soil: sand)). After the soil was air-dried, loosened, homogenized, and sieved in 2 mm net sieve, acidity was corrected by applying dolomite clay, increasing base saturation up to 60%, according to recommendation by Prezotti, Gomes, Dadalto, and Oliveira (2007) for green leaf cultivation in the state of Espírito Santo. Following limestone addition, the vases were submitted to a period of twenty-one-day incubation.

The experiment was set up in a factorial scheme 5 x 2, with 5 doses of phosphorus and 2 lettuce cultivars in a completely randomized design, with four repetitions. Phosphate fertilization was performed at the end of the incubation, with the phosphorus levels in the treatments being: 0, 100, 200, 300, and 500 mg dm^{-3}. The P doses and fertilization procedures were calculated and carried out according to methodology proposed by Novais, Neves and Barros (1991) for vase fertilization. Phosphorus was added in the form of potassium dihydrogen phosphate p.a. (KH_2PO_4). The seedlings of the Lisa and Americana lettuce varieties were transplanted after these procedures.

Nitrogen and potassium fertilizations were applied under cover top, 15 days after transplanting, according to recommendation by Novais, Neves and Barros (1991). Ammonium sulfate p.a. ($(NH_4)_2SO_4$) was used as a source of nitrogen, and potassium sulfate p.a. (K_2SO_4) was used as a source of potassium, so as to complement this nutrient's dose supplied by the source utilized for phosphorus. Daily irrigations were carried out up to a day before harvest, 40 days after seedling transplanting.

The lettuce plants were separated at the stem base for a separate analysis of the aerial part and roots, which were washed with distilled water, packed in individual paper bags previously identified and placed inside a forced air circulation stove at a temperature of 70ºC for 48 hours for drying and dry mass determination. The dried material was ground in a Willey type mill to determine the phosphorus content of the aerial part and roots, which, in turn, was colorimetrically obtained using an UV-visible spectrophotometer, by the method of phosphomolybdate reduction by ascorbic acid, as described by Embrapa (2009).

The following characteristics were evaluated: (a) aerial part dry matter (APDM); (b) root dry matter (RDM); (c) phosphorus content of the aerial part (PCAP); (d) phosphorus content of the root (PCR); (e) phosphorus accumulation of the aerial part (PAAP), obtained by dividing between dry matter and phosphorus content of the aerial part; (f) phosphorus accumulation of the roots (PAR), obtained by dividing between dry matter and phosphorus content in the root; (g) phosphorus absorption efficiency (PAE), obtained by dividing between total phosphorus accumulation and root dry matter; (h) phosphorus translocation efficiency (PTE), obtained by dividing between phosphorus accumulation in the aerial part and total phosphorus content; (i) phosphorus use efficiency in the aerial part (PUEAP), obtained by dividing between aerial part dry matter and phosphorus content in the aerial part; and (j) root/aerial part (R/AP), obtained by dividing between root dry matter and aerial part dry matter

The results were submitted to analysis of variance (ANOVA) and to the Tukey test, with regression test being applied for the significant values at 5% probability. The statistical analysis was carried out using the computational program SAEG 9.1 (Federal University of Viçosa-UFV, 2007).

3. Results and Discussion

Root dry matter (RDM) and root-aerial part (R/AP) presented a significant interaction ($p < 0.05$) between the two varieties and the P doses applied (Table 3). On the other hand, the other characteristics evaluated did not show a significant interaction, with some presenting the factor variety as significant (Table 4), and others the factor P dose (Table 5). Thus, for these characteristics, the factors were studied separately.

Table 3. Interaction of Lisa and Americana lettuce varieties with the P doses for the characteristics root dry matter (RDM) and root-aerial part ratio (R/AP)

Variety	Phosphorus doses (mg dm^{-3})				
	0	100	200	300	500
	RDM (g)				
Lisa	1.04 Aab	1.13 Aa	0.72 Abc	0.49 Ac	0.44 Ac
Americana	0.53 Ba	0.31 Ba	0.38 Ba	0.37 Aa	0.39 Aa
	R/AP (g g^{-1})				
Lisa	0.41 Aa	0.34 Aa	0.21 Ab	0.15 Ab	0.14 Ab
Americana	0.21 Ba	0.09 Ba	0.12 Ba	0.10 Aa	0.11 Aa

Same upper-case letters in the column and lower-case letters in the line do not differ at 5% probability by the Tukey test.

The analysis of variance indicated a significant difference in RDM and R/AP in relation with the doses used in the Lisa variety (Table 3), which, at the dose of 100 mg dm^{-3}, presented a RDM significantly higher than the others, not differentiating only from the control. Consequently, R/AP was superior at the two lowest doses (0 and 100 mg.dm^{-3}). The varieties differed at the three lowest doses studied, with Lisa being superior in the two characteristics evaluated, showing that the amount of phosphorus in its root is higher than in the Americana. Marschner (1995) emphasizes that the genotypic differences are related to P absorption, transport and utilization inside the plant, which are affected by morphological and physiological factors, as well as by the plant's nutritional demand.

Table 4 shows that Americana was superior in the three characteristics showing significance (PCAP, PAAP and PAE), showing it presents a higher absorption of P, with most of it being found in its aerial part.

Table 4. Characteristics evaluated for the Lisa and Americana varieties

	Varieties	
Characteristics	Lisa	Americana
PCAP (g kg^{-1})	1.12 b	1.19 a
APDM (g)$^{n.s}$	3.09 a	3.25 a
PAAP (mg)	3.53 b	4.00 a
TPR (g kg^{-1})$^{n.s}$	1.54 a	1.38 a
PAAP (mg)$^{n.s}$	1.25 a	0.56 a
PAE (mg g^{-1})	7.86 b	12.23 a
PTE (mg g^{-1})$^{n.s}$	0.24 a	0.27 a
PUEAP$^{n.s}$	3.28 a	3.43 a

Same letters in the line do not differ at 5% probability by the Tukey test. $^{n.s}$Values were non-significant at 5% probability by the F test.

Phosphorus content in the aerial part (PCAP) was affected by the amount of P applied (Table 5), increasing significantly with P dose increase, and being higher at the dose of 500 mg dm^{-3}. Due to PCAP contribution for PAAP calculation, this characteristic followed the same pattern observed previously, showing no difference only between treatments 100 and 200 mg dm^{-3}.

Phosphorus Absorption Efficiency (PAE) also increased with dose increase, with the highest efficiency occurring at the two highest doses, with no significant difference between them.

Table 5. Characteristics evaluated for the phosphorus doses

	P doses (mg dm^{-3})				
Characteristics	0	100	200	300	500
PCAP (g kg^{-1})	0.48 d	0.78 cd	1.11 bc	1.48 b	1.92 a
APDM (g)	2.54 b	3.30 a	3.23 a	3.46 a	3.32 a
PAAP(mg)	1.22 d	2.57 c	3.55 c	5.08 b	6.40 a
PAR (g kg^{-1})$^{n.s}$	1.21 a	0.93 a	1.85 a	1.99 a	1.31 a
PCR(mg)$^{n.s}$	1.04 a	0.82 a	1.29 a	0.80 a	0.56 a
PAE (mg g^{-1})	2.98 c	6.23 bc	9.61 b	14.30 a	17.11 a
PTE(mg g^{-1})$^{n.s}$	0.23 a	0.25 a	0.26 a	0.25 a	0.28 a
PUEAP	5.41 a	4.26 ab	2.99 bc	2.38 c	1.73 c

Same letters in the line do not differ at 5% probability by the Tukey test. $^{n.s}$Values were non-significant at 5 % probability by the F test.

The aerial part dry mass (APDM) was statistically lower at dose 0 mg dm^{-3}, presenting no significant difference in the other treatments, influencing phosphorus use efficiency in the aerial part (PUEAP), which was significantly higher at the two lowest doses, decreasing with dose increase. This fact was due to DMAP constancy and to PAAP growing increase, according to the doses. The characteristics PCR, PAR, and PTE presented no significant difference among the P doses used in the experiment. Fresh and dry matter production by the plant, P absorption and use efficiencies are influenced by the use of micronutrients (Yuri et al., 2002). Micronutrients, such as boron and zinc, influence P absorption at different dosages, as verified by Moreira, Fontes and Camargos (2001) showing the importance of studies on P absorption in the presence of micronutrients.

P absorption efficiency increased for the two lettuce varieties (R^2 = 0.96 and 0.98, respectively) with P dose increase. Decreased absorption tended to occur above the dosage of 400 mg dm^{-3} for the Americana variety, which presented a higher P absorption efficiency than the Lisa variety. The latter had a lower P absorption efficiency which increased linearly with P dose increase (Figure 1).The aerial part dry matter of the Americana variety increased exponentially ($R^2 = 0.87$) with P dose increase.

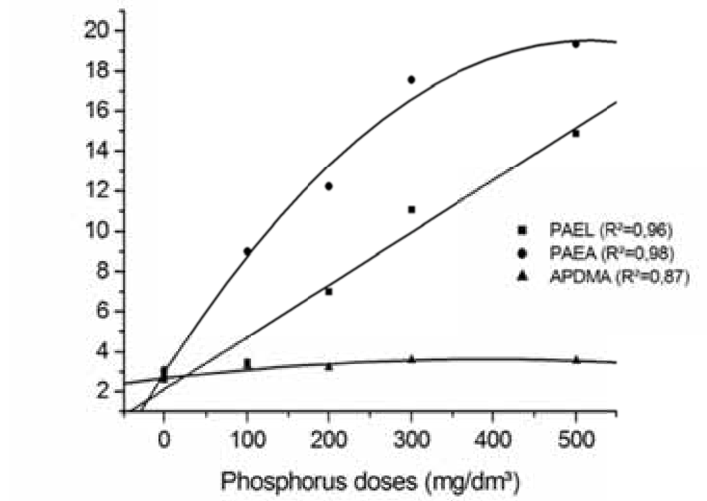

Figure 1. Mean values of phosphorus absorption efficiency of the Americana and Lisa varieties (PAEA and PAEL), respectively, and aerial part dry matter of the Americana cultivar (APDMA)

Mota et al. (2003) verified that the Americana variety presents a larger cycle of phosphorus development and extraction than the lettuce belonging to the group of smooth or curly leaves, demanding more P than other varieties. According to Prado (2008), P is intimately linked to the synthesis of amino acids and carbohydrates in the plant, and its deficiency leads to reduced matter production. P content in the aerial part of the Lisa and Americana varieties increased with phosphorus dosage (R^2 = 0.99 and 0.99, respectively). However, P use efficiency by the aerial part of the two varieties decreased exponentially (R^2 = 0.98 and 0.99, respectively), with phosphorus dosage increase (Figure 2). This may be related to the number of days the plants were cultivated (40 days), which may not have been sufficient to meet their nutritional needs (Mota et al., 2003).

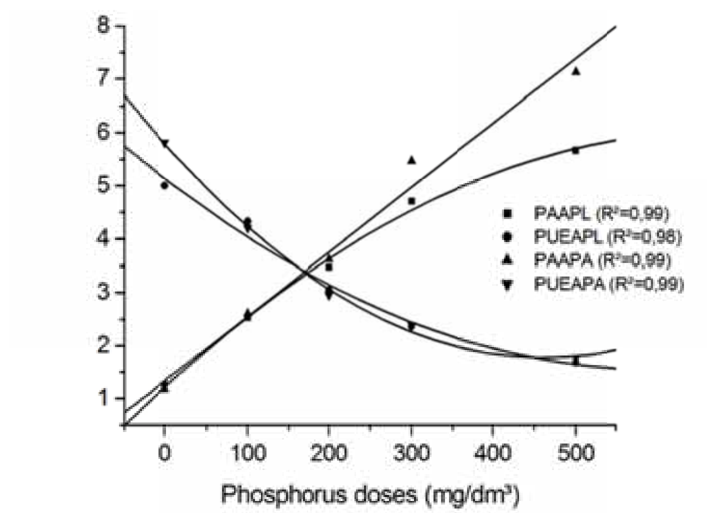

Figure 2. Mean values of the phosphorus accumulation in the aerial part (PAAPL and PAAPA) and phosphorus use efficiency by the aerial part (PUEAPL and PUEAPA) by the Lisa and Americana varieties, respectively

Phosphorus translocation in the aerial part increased linearly for the two varieties (R^2 = 0.99 and 0.99, respectively). Above the dosage of 150 mg dm^{-3}, translocation was higher in the cultivar Americana than in the Lisa, with increased P dosage (Figure 3). P translocation efficiency by the variety Lisa had an exponential increase (R^2 = 0.99) with increased P dosage.

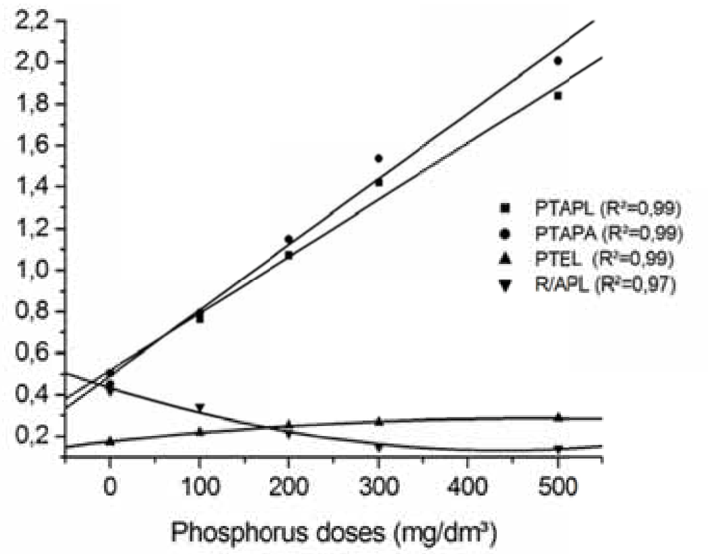

Figure 3. Mean values of phosphorus translocation in the aerial part (PTAPL and PTAPA) of the varieties Lisa and Americana, respectively, and phosphorus translocation efficiency (PTEL) and root/aerial part (R/APL) of the variety Lisa

On the other hand, root/aerial part of the Lisa variety decreased exponentially (R^2 = 0.99) with P dosage increase (Figure 3). This shows that this dosage promoted differences in root and aerial part absorption, a fact also verified by Cock et al. (2002, 2003) and Mota et al. (2003), when evaluating the nutritional efficiency of phosphorus in lettuce.

4. Conclusions

- Root dry matter (RDM) and root/aerial part(R/AP) were influenced by the interaction between the varieties and the P doses, with Lisa presenting the highest values of these characteristics at the lowest doses evaluated.

- The characteristics PCAP, CONTPA and EAP presented a significant difference between the varieties, with Americana presenting the highest values.

- The characteristics PCAP, PAAP, PAE, APDM, and PUEAP presented a significant difference between the P doses, with the highest values being found at dose 500 mg dm^{-3}, except for PUEAP, which was 0 mg dm^{-3}.

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Assessing Environmental Damage to Marine Protected Area: A Case of Perhentian Marine Park in Malaysia

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Abstract

The *Perhentian* Island located in the East coast of Peninsular Malaysia is well-known for its rich coral reef ecosystems. Marine resources of Malaysia have been overexploited due to overfishing and tourism activities. As such no-take marine protected area (MPAs) were established in Malaysia, including *Perhentian* Island Marine Park to enable overexploited marine resources to recover and to conserve coral reef ecosystems. This paper examines the current level of activities causing damage to coral reef habitats in the *Perhentian* MPA. This study used paired comparison method to elicit the perception of local stakeholders on activities harmful to the marine habitats. The results of the analysis showed that various respondent groups had similar preference rankings on the harmful activities: littering, discarding fishing equipment, excess fishing and too many divers that cause damage to habitats in the MPA area. The findings suggest that policy makers should take cognizance of the local stakeholders' concern in planning and designing of marine protected areas in Malaysia.

Keywords: marine protected areas, tourism, overfishing, preference ranking, Peninsular Malaysia

1. Introduction

Marine Protected Areas **(**MPAs) have been suggested as an important tool for fishery management particularly in a situation where fisheries are overexploited (Alban & Boncoeur, 2006). In Malaysia the government has undertaken MPA programmes in offshore islands in the 1980s. Malaysia has 3,600 km^2 of coral reef areas which are mostly protected as marine parks. The primary objective of establishing MPAs is to protect coral reef ecosystems from fishing and other harmful activities to ensure sustainable benefits from fisheries and tourism services. In Malaysia massive tourism development has taken place over the last decades and this sector accounts for the country's second largest source of foreign exchange earnings. Several studies highlight that MPAs allow tourism activities to enhance economic benefits while enabling the recovery of overexploited marine resources (Kelleher & Bleakley, 1995; Lauck et al., 1998; Gue´nette & Pitcher, 1999; Russ & Alcala, 2003; Gaylord et al., 2005).

Perhentian island is one of the coral reef islands in the east coast of Peninsular Malaysia. This island has been gazetted as a Marine Park in 1994. Various infrastructures have been developed in the island to promote tourism. In *Perhentian* island statistics on tourist arrivals showed an increasing trend from 51,000 in 2004 to 90,000 in 2011 (Department of Marine Park, 2012). Several studies highlight that the coral habitats in *Perhentian* Island has been damaged due to expansion of tourism activities (Harborne et al., 2000; Tamblyn et al., 2000). Other studies also show that Malaysian coral reefs are under medium to high levels of threat due to human activities (Burke, 2002). In *Perhentian* Island the live coral cover was the lowest (36.5%) compared to other islands in Peninsular Malaysia (Reef Check Malaysia, 2011). Studies outside Malaysia showed that the increasing tourism activities especially diving and snorkeling in marine parks are the main reasons for deteriorating coral reef health (Ward, 1990; Hawkins & Roberts, 1993; Davis & Tisdell, 1995; Rouphael & Inglis, 2001). In Malaysia the main reasons for coral degradation are due to the large number of tourists visiting this island, construction of land based tourism infrastructures, fishing, and pollution through waste disposal and littering (Reef Check Malaysia, 2011).

The negative consequences of human activities to coral reefs have not been given due consideration in formulating the tourism management policy in Malaysia. Wattage et al. (2011) found that the major threat to coral reef comes from human activity which has not been addressed in the management of MPA. In this paper, we elicit community perceptions to determine the most harmful activities in Perhentian MPA that damage coral reefs using paired comparison method. The results of the study will be useful for policy makers to formulate effective planning and management of MPAs in Malaysia.

This paper is organized as follows: Section 2 describes the general characteristics of *Perhentian* MPA and its current management status. Section 3 presents the method of paired comparison, questionnaire and data sources. Section 4 discusses the demographic characteristics of respondents; Section 5 discusses the results, while Section 6 presents policy formulations for MPA and the final section presents the conclusions.

2. *Pulau Perhentian* Marine Protected Area (PPMPA)

The *Pulau Perhentian (Perhentian Island)* is located in the South China Sea, 21 km off the mainland of Terengganu State in the east coast of Peninsular Malaysia. The island is easily accessible by speedboat from the small fishing port of *Kuala Besut* on the mainland. The *Perhentian* Archipelago consists of two main islands, *Perhentian Besar* (large) and *Perhentian Kecil* (small), with an approximate land area of 867 and 524 hectares, respectively (Figure 1). There are also several smaller islands located close to the *Perhentian Kecil* island. With a diverse coral reef ecosystem and inter-tidal habitats, *Pulau Perhentian* is a breeding, nursing and feeding ground for numerous fish species, sea turtles and other resources. Tamblyn et al. (2005) conducted a study on coral reefs of Malaysia. They have recorded 127 types of fish species available in the marine waters surrounding *Pulau Perhentian.*

Marine protected area (MPA) of *Pulau Perhentian* was first initiated by the Department of Fisheries Malaysia (DoFM) in 1983. This island was gazetted as a Marine Park in 1994 and then established as a no-take MPA where fishing was prohibited within two nautical miles from the lowest water level in the shore. In 2004, the responsibility of MPA management was transferred from DoFM to the Marine Parks Department Malaysia (MPDM) under the Ministry of Natural Resources and Environment (MNRE). The main reason for this arrangement was to promote sustainable resource management and enhance tourism in the marine parks. However, both DoFM and MPDM belonged to the Federal government jurisdiction, while the land in the island belongs to the State Government, which decides on all land based development activities. Coordination between state and federal governments is important for sustainable resource use in the island. However, there is a dichotomy in jurisdiction between federal and state governments in Malaysia. Gopinath and Puvanesuri (2006) highlighted that the main constraint for successful marine resource management was the lack of coordination.

The government has undertaken legal protection in order to protect coral reefs from fishing in the MPA area. However the legal protection has been less effective in *Perhentian* island due to some reasons. Firstly, local residents who primarily rely on fishing for their livelihoods are not allowed to fish in the no-take MPA area. Although, tourism activities has provided economic opportunities for the local people, but fishers still depend on fishing in the area for their subsistence. Secondly, the government has limited capacity to control illegal fishing due to lack of enforcement of fishing rules particularly during monsoon seasons (October-February) when majority of the households participate in fishing in the MPA area.

A massive physical infrastructural development has also taken place in *Pulau Perhentian* over the last decade. These infrastructures consist of three jetties, a primary school, a health clinic, a police station and a post office. A generator was installed in 1994 for electricity supply; and a water treatment plant was established to provide piped water in the village. There are about 45 resorts/chalets, 19 dive shops, 40 souvenir shops, several restaurants and tea stalls employing a good number of local residents in *Perhentian* besar and *Perhentian kecil*. For the small area of *Perhentian* Island, the number of infrastructures was relatively high compared to other marine parks in the east coast of Peninsular Malaysia.

There is a small village called *Kampung Pasir Hantu* of approximately 10 ha situated in *Perhentian kecil* (small island). A total of 1,500 residents live in this village, and all of them are Malays. Most of the local residents were fishers prior to the establishment of *Perhentian* MPA. The villagers have adopted various tourism activities for better income. The main tourism activities include SCUBA diving, snorkeling, swimming and recreational fishing. These activities are carried out from March to September. During the northeast monsoon season (October to February), majority of local people participate to fish in *Perhentian*. All kinds of tourism activities are closed during this period. Fishers use artisanal fishing gear, namely hook and lines, traps, gill and drift nets. Trawls with relatively smaller boats operate at short distances from the coast. Fishers from other neighboring areas are also engaged in fishing in this area during this season.

Figure 1. *Perhentian* island and Marine protected area

3. Methodology

The study adopted a primary survey approach to obtain data from the selected respondents in *Perhentian* island. The study investigated on how the coral reefs were being damaged in the *Perhentian* island MPA. This study provides information that helps to develop guidelines for management to protect and conserve coral reefs in *Perhentian* island.

3.1 Paired Comparison and Damage Schedule Approach

The study used the 'damage schedule' method developed by Chuenpagdee (1998). This method was used to elicit public judgments on identifying the most important harmful activities damaging coral reef ecosystem in *Perhentian* MPA. This method has been used to examine coastal environmental problems in Southern Thailand and Mexico (Chuenpagdee, 1998; Chuenpagdee et al., 2001, 2002). Quah et al. (2006) used this method to examine the environmental damage in urban coastal areas in Singapore. They found that the paired comparison method was suitable to develop a schedule that policy makers can use as a guide to reduce environment problems in coastal areas. Chuenpagdee et al. (2001) highlights that the damage schedule method is more suitable and transparent compared to other non-market resource valuation methods.

The paired comparison approach presents two scenarios at a time. The total number of possible pairs for all scenarios is $N(N-1)/2$, where N is the number of scenarios. For each pair, given a specific situation, respondents were asked to choose only one scenario (either X or Y) that they considered as more important. Each selected scenario was scored and aggregated for analysis. These scores were normalized to a scale of 0 (least importance) to 100 (highest importance), and ranks were assigned based on the scale values. This assessment helps to develop a damage schedule or a predetermined schedule to revise current MPA management practices. This method guides policy makers in formulating policy design, regulatory control, management choice and payment for damages (Chuenpagdee et al., 2001).

3.2 Questionnaire and Data Source

The data for this study was obtained from face-to-face interview with selected respondents using structured questionnaire. Prior to field data collection, focus group discussions (FGD) and key informant surveys (KIS) were carried out to gather information about the status of coral reefs, livelihood options of local people, and tourism management in *Perhentian* MPA.

The questionnaire consists of three sections: Section A included household characteristics, income and employment; Section B included perception on the status of coral reefs, and information exchange among the local people; Section C included paired comparison questions comprising seven activity scenarios that damage coral reefs in the first set (set A) and seven community programmes in the second set (set B) giving a total number of 21 pairs for each set (Table 1). A total of 42 pairs from two sets were included in the survey booklet. A list of harmful activities for damaging coral reefs and several conservation oriented community programmes were identified from FGD and KIS. As shown in Set A of Table 1, the important harmful activities were fishing, diving, littering in the MPA. In Set B, important community programmes i.e., establishing artificial reefs, microcredit support and providing secure access to fishing and tourism activities are included.

The study investigated the severity of harmful activities in damaging coral reefs in *Perhentian*. In the first set (Set A, Table 1), respondents were asked to select only one resource damage activity from each pair that they considered more "severe" in terms of the coral damage in the MPA. In the second set (Set B, Table 1), respondents were asked to choose one community programme that was likely to benefit the community and provide incentives to promote conservation.

Table 1. Coral damage activities and community programmes

Set A: Activity scenarios for coral damage
• Many people fishing in MPA area
• Fishing in spawning area in MPA
• Fishing using hook and line
• Too many motorized boats used by tourists
• Diving in the coral reef area in the MPA
• Littering on the beach
• Discarding fishing equipments
Set B: Community programme
• Establish artificial reefs for fishing
• Provide technical training to the fishers
• Provide micro credit
• Establish aquaculture in the sea
• Provide fishing access for subsistence
• Secured employment in tourism
• Reduce the MPA area

Prior to the survey, a complete household census was conducted in *Perhentian*. Respondents were divided into four subgroups, according to their main occupation, i.e., (1) fishers; (2) tourism workers such as boat operators, tourist guide, (3) traders/ shop owners; (4) other local residents such as government employees, housewives, retired persons. A separate respondent group or "Expert group" with experience in marine resources in this area was identified thorough discussions with various agencies in Terengganu. The respondents of this group comprised of staff from government departments, universities, and non government organizations (NGO). Quota sampling was used to select a minimum of 30 respondents from each of the sub groups. A total of 128 respondents were selected for interviews from the five sub groups. The selected respondents were informed in advance about the interview with the assistance of the village leader and local enumerators. Interviews were mostly conducted in late afternoon as most of them were available during this time. The survey booklet was completed through face to face interview. A map of the island, a set of pictures and fact sheets describing the status of coral reefs and fisheries resource conditions in *Perhentian* were provided to each respondent during the survey. Each survey took around 45 minutes to complete.

4. Respondents Characteristics and Their Perceptions Towards Resources in MPA

4.1 Demographic Characteristics

The study interviewed a total of 128 respondents of which 107 were males, while 21 were females. Among the five subgroups, tourism workers and traders were relatively younger (average age below 40 years) compared to fishers (48 years), experts (45 years) and others (48 years). The average household size was 6, ranged from 3 to 9 people. Majority of the tourism workers and traders had attained secondary education, while about half of the fishers had attained primary education.

Table 2. Respondents perception on resource condition in MPA (%)

Resource	Declining	Increasing	Same	Don't know	All
Beach	42.9 (45)	40.0 (42)	17.1 (18)	-	100 (105)
Coral Reef	49.7 (53)	37.7 (39)	9.7 (10)	2.9 (3)	100 (105)
Fish biomass	48.5 (51)	43.8 (46)	4.8 (5)	2.9 (3)	100 (105)

Note: number of respondents is shown in parentheses; the expert group is not included here.

About half of the respondents informed that the condition of coral reefs and fish biomass has severely deteriorated in *Perhentian* Island MPA (Table 2). However, the deterioration is less severe in sandy beaches. The evidence suggests that the declining of coral reefs was due to the increasing number of tourist activities in this island.

Table 3. Source of information exchange in *Perhentian* island

Agency/person	Number of respondents	%
Village Leader	35	33.7
Department of Fisheries	34	33.2
Department of Marine Park	84	80.8
Friends/relatives	46	44.2

The study shows that the majority of respondents (81%) had access to information regarding MPA management from the local marine park office. About 44% of respondents reported that they obtained information from their friends and relatives. The results indicate that there is a lack of communication support from fisheries agencies in the marine park.

Table 4. Kendal Tau rank-order correlation coefficients

Group	Fishers	Tourism workers	Traders	Others	Experts
Resource damage activities					
Fishers	1.000				
Tourism	0.964**	1.000			
Traders	0.964**	1.000**	1.000		
Others	0.929**	0.964**	0.964**	1.000	
Experts	0.752*	0.857**	0.857**	0.786*	1.000
Community Programmes					
Fishers	1.000				
Tourism	0.881**	1.000			
Traders	0.821**	0.992**	1.000		
Others	0.672	0.870**	0.871**	1.000	
Experts	0.909**	0.988**	0.975**	0.821**	1.000

** Denotes significant correlation at $p = 0.01$.

*Denotes significant correlation at $p = 0.05$.

5. Paired Comparison Analysis Results: Community Judgment about MPA

Individual respondents' preference scores were obtained through paired comparison survey. These scores were aggregated for each sub-group (fishers, tourism workers, traders, experts, and others) and ranks were assigned to these aggregated scores. The Kendall's Tau rank-order correlation analysis was used to test if there was an agreement in the rankings provided by the respondent groups. As shown in Table 4, the rankings for the five sub groups were close-to-perfect correlations. All the Kendall's Tau rank-order correlation coefficients were significantly less than the *p*-value of 0.05. This indicates that the level of agreement was highly correlated among respondent groups, in both resource damage activities and community programmes.

Table 5 presents the aggregated performance score for the activity scenarios and community programmes based on the paired comparison for each of the five subgroups. The score values were normalized (0 to 100) and a ranking was assigned to these normalized scores in order to test for an agreement between the respondent groups using Kendall coefficient of agreement (u) (Siegel and Castellan, 1988). This test measures the degree of similarity of rank ordering provided by a single respondent group. The value of u is one when the level of agreement is full among individuals. The chi-square goodness-of-fit test was also used to test the significance of agreement for a large sample size. The chi-square goodness-of-fit tests for Kendall coefficient of agreement (u) were all less than the critical p – value of 0.001 level of probability, thus rejecting the null hypothesis that significant agreement exists among the respondents in each subgroup (Table 5).

Littering and discarded fishing equipment were found to be the most harmful activities to coral reefs. The range of the scale value between these two activities was relatively large for the expert group (58 to 93) but the range was smaller for the other four groups (Table 5). The results of the study suggest that the expert group is not fully aware of the problem of discarding fishing materials, although they are concerned about fishing in spawning area (scale value of 72).

Table 5. Aggregated performance scores for activities and programmes by groups

Damage activity and community programmes	Fishers	Tourism	Traders	Others	Experts
Resource damage activities					
Littering on the shore	75 (1)	85 (1)	83 (1)	87 (2)	93 (1)
Discarded fishing equipments	72 (2)	81 (2)	73 (2)	88 (1)	58 (3)
Fishing in spawning area	65 (3)	60 (3)	55 (3)	45 (3)	72 (2)
Too many people fishing	32 (5)	48 (4)	44 (4)	43 (4)	55 (4)
Diving in the coral reef area	58 (4)	30 (5)	29 (5)	29 (5)	20 (7)
Too many motorized boats	19 (6)	14 (6)	23 (6)	24 (6)	29 (5)
Fishing using hooks and line	12 (7)	13 (7)	16 (7)	18 (7)	19 (6)
Number of respondents k	30	32	29	14	23
Kendall coefficient of agreement u	0.448	0.496	0.352	0.304	0.315
Chi-square*	293.6	343.9	227.8	104.0	166.5
Community programmes					
Establish artificial reefs	77 (1)	71 (1)	62 (1)	61 (3)	83 (1)
Provide technical training to fishers	59 (2)	70 (2)	61 (2)	85 (2)	71 (2)
Provide micro credit to fishers	54 (3)	68 (3)	59 (3)	76 (1)	64 (3)
Establish aquaculture in the sea	52 (4)	48 (4)	51 (4)	43 (5)	46 (4)
Provide fishing access	31 (6)	31 (5)	34 (6)	36 (6)	36 (6)
Building residence for tourists	31 (6)	39 (5)	42 (5)	48 (4)	43 (5)
Reduce fishing restriction	44 (5)	22 (7)	27 (7)	23 (7)	23 (7)
Number of respondents	30	32	29	14	23
Kendall coefficient of agreement u	0.370	0.469	0.551	0.314	0.224
Chi-square*	168.8	208.0	247.2	138.6	153.2

*significant agreement at p <0.001.

Note: Figures in parenthesis show rankings.

In the community programmes, it was found that fishers mostly preferred the activities that might increase fish productivity (scale value of 77) for example, increase fish biomass through artificial reef establishment, while other groups (women and traders) preferred micro credit programme (Table 5). Local people can use micro credit to invest in various self employment activities to increase their income. Thus, dependency on marine resources for livelihoods may be reduced.

The study found that both the resource dependent group and expert group had similar concerns about harmful activities to coral reefs. The respondent groups preferred similar community programmes in the *Perhentian* MPA. Therefore, it was possible to aggregate the scores of all respondents into one scale for damaging activities and another scale for community programmes (Table 6). Among the activities, littering was found to be the most damaging activity (scale value of 85), followed by discarded fishing equipments (scale value of 74).

Table 6. Aggregated performance scores

Coral Damage Activities	All groups	Community Programme	All groups
Littering on the shore	85(1)	Creating more artificial reefs in the MPA	71 (1)
Discarded fishing equipments	74(2)	Build a centre for technical training	69 (2)
Fishing in spawning area	59(3)	Provide micro credit support	64(3)
Too many people fishing	44(4)	Promote small scale aquaculture	48 (4)
Diving in the shallow part of MPA	33(5)	Build hotels/chalet for tourists	41(5)
Too many motorized boats	22(6)	Provide fishing access for local fishers	34(6)
Fishing using hooks and line	16(7)	Reducing the restricted area of MPA	28 (7)

6. Policy Implications for MPA Management

The results of the study showed that all the sub groups of respondents (fishers, tourism workers, traders, experts and others) were concerned about littering and fishing activities damaging coral reefs in *Perhentian* MPA. The respondents had suggested the most damaging activities and the least damaging activities to coral reefs. Based on the community judgment it is possible to develop predetermined damage schedule that can be used as a guide to adjust the MPA management policy. Policy makers can impose sanctions as well as incentives for the activities in the MPA for sustainable use of coral reef ecosystems in *Perhentian* island.

The existing policy on tourism management does not effectively protect the coral reefs in *Perhentian* Island. The increasing number of tourists visiting this marine park poses pressure on the island. The beach and coral reefs are excessively used by the visitors undertaking various tourism activities. The current use of coral reefs should be reduced and the number of tourists should be regulated based on the carrying capacity of the coral reef habitat.

The increasing number of chalets and jetties negatively affects coral reef ecosystems. Development agencies should assess the environmental impact of infrastructures on the environment of the MPA. Studies have highlighted that small island destinations are the most vulnerable to environmental impacts from tourism activities (Gossling, 2003). Salmond (2010) found that land based development activities had created significant damage to the coral reef habitats in *Perhentian* Island. The environmental consequences of tourism in the marine parks should be addressed in the design and implementation of marine policies in Malaysia. There is a need to conduct Environmental Impact Assessment (EIA) to assess the possible risk and threats from these activities on the resources in the MPA.

Although fishing is totally prohibited in the MPA area in *Perhentian*, there is clear evidence that the majority of the local fishers are involved in fishing particularly in the monsoon season. Fishing nets are entangled with coral reefs which hinder diving activities as well as pollute the coral reef habitats. Prior to imposing no take MPA, local fishers were not adequately consulted. Participation of local fishers in the MPA management is essential since the government has limited capacity to enforce management regulations. Effective coordination between the important agencies such as the Department of Fisheries and Department of Maine Parks is required to ensure fishers cooperation and support to protect coral reefs in the MPA.

In Malaysia the state government has authority for all land management in the marine park islands, while the Marine Park Department Malaysia (MPDM) is responsible for the management of MPAs water area up to two nautical miles surrounding the island. These overlapping jurisdictions of legislation between state and federal governments are the major constraint for preserving coral reef resources in *Perhentian* MPA. The top down management system in Malaysia generally excludes local people's participation in the management of coastal marine resources. There is also lack of coordination between the federal and state governments.

The government should restrict land development activities, particularly in the land scarce small island such as *Perhentian*. Both state and federal agencies should give priority to involve the community which is a key factor in ensuring the success of a marine protected area (White, 1986; Kaza, 1988; Kenchington, 1988; Cinner, 2005). Sumaila et al. (2000) highlighted that successful implementation of MPA management is difficult without consultation with the local people.

The results of the study revealed that local people are aware of the damaging activities in the *Perhentian* island. The government should provide public awareness and education programs to the community. These programmes help the community to understand the importance of protecting coral reef ecosystems in the marine parks. Besides the community, marine park staff, tour operators, dive operators should also be adequately trained to reduce harmful activities and ensure sustainable management of MPAs in Malaysia.

7. Conclusion

The results of the study provide scales of various damaging activities which can be used to construct a damage schedule to be used as a policy guide for protecting coral reefs in *Perhentian* MPA. This schedule is based on the community agreement on how coral reef ecosystems are negatively affected. The schedule can impose more strict sanctions on the severe harmful activities and impose less strict sanctions on the less harmful activities. The study reveals that the severe damaging activities are due to the overwhelming pressure from tourism activities on the small island. The current tourism policy does not effectively protect the coral reef ecosystems. The government can reduce the use of coral based recreational activities such as diving and snorkelling in *Perhentian* MPA.

Sustainability of tourism in this island depends directly on the health of coral reefs. Excess fishing is also harmful to the coral reefs; however, fishers primarily depend on fishing for their livelihoods. It was expected that fishers would shift to tourism activities to compensate their loss of income from fishing which has been restricted in the MPA area. The fisher respondents had suggested implementing artificial reefs surrounding the MPA area where they can fish. Such incentives may foster conservation of coral reef habitats and achieve long term benefits from MPA. Credit support can also promote local people who can invest in other non fishing income opportunities. The community development programmes suggested by the local people is important for policy makers to formulate conservation based planning and management of the marine parks in order to reduce exploitation of fisheries and coral reefs.

Community awareness towards protecting coral reefs is vital in *Perhentian* as this resource is the dominant contributor to the livelihoods of local residents. Various community awareness programmes should be conducted at the local level to inform the users about the importance of protecting and conserving coral reefs for their sustainable benefits. These awareness programmes will help to improve knowledge among the users. The most important concerns about coral reef damage need to be discussed between the users and the government authority. Thus a consensus can be made through such discussions and help formulate sustainable management of the coral reef resources in the MPA in *Perhentian* island.

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Selection of Black Bengal Buck Based on Some Reproductive Performance of Their Progeny at Semi-Intensive Rearing System

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Abstract

The objective of this study was to select Black Bengal Buck Based on some reproductive performance of their progeny. The least-squares means for overall reproductive performances of age of first kidding (AFK), weight at first kidding (WFK), gestation length (GL), kidding interval (KI), post-partum heat (PPT), litter size at birth (LS) and litter weight at birth were 465.6 days, 13.22 kg, 145.34 days, 302.5 days, 123.84 days, 1.61 and 1.66 kg, respectively. The effect of flock and generation were significant ($p<0.05$) for AFK, WFK, KI, PPT, LS and LWB. The effect of parity of doe was significant ($p<0.01$) for LSB and LWB. The effect of season was significant ($p<0.05$) for KI, PPT and LWB. The heritability value for these traits was ranges from 0.17 to 0.24 and predicted breeding value from -0.003 to 0.445. According to the genetic worth of the buck the highest breeding value of reproductive traits were found in the progeny of Buck No. 32, followed by Buck No. 52, 54, 81 and 87. This progeny tested bucks may be used for the improvement of the reproductive potentials of Black Bengal goat through Artificial Insemination (A.I). The lowest breeding value of reproductive traits was found from the progeny of Buck No. 3 followed by Buck No. 11. The low estimates of heritability obtained for reproductive traits indicated that selection based on the doe's own performance may result in slow genetic improvement therefore; the progeny testing program will be beneficial to the farmers and fulfill their need by supplying superior sires of high genetic merit.

Keywords: black bengal buck, reproduction, progeny, breeding value, selection

1. Introduction

Reproductive efficiency is always considered to be the most vital factor ensuring increase in productivity to a certain environmental condition (Hossain et al., 2004). Increased production efficiency can be obtained from goats since they have a high reproductive efficiency with the potential for increased litter size and shorter generation interval and they have a relatively higher fertility rate in comparison to other farm animals (Williamson & Paney, 1978). Reproductive performance of goats is a major determinant of productivity and economic viability of commercial goat farms. The goats' reproductive performance is an indicator of their adaptation to the adverse conditions. Reproduction is a complex composite trait influenced by many components including puberty, estrus, ovulation, fertilization, embryo implantation, pregnancy, parturition, lactation, and mothering ability. Reproductive efficiency in female goats is determined by many different processes (Shelton, 1978). These processes include, for example, the length of the breeding season, cyclic activity, ovulation rate, fertilization rate, the post-partum anoestrous period and the growth and viability of the offspring.

Selective breeding using superior buck expected to improve the productive efficiency of Black Bengal goats without much alteration in the prevailing production system. Selective breeding was an important tool for conservation of the well adapted genetic resources and thus avoid fore coming extinction of the breed (Dhara et al., 2008). Selection improves the reproduction and growth for conserving the characteristics to adopt tropical goats to their environment and reduce under harsh environmental conditions and have good fitness traits (Wilson, 1989).

Reproductive traits are economically important characters which could be improved by selection of local breeds (Mourad, 1993). The number of individuals born per parturition makes a much greater contribution to the total weight of individuals weaned than the growth rate of individuals (Bradford, 1985).Thus reproductive rate is an economically important trait in small ruminant production enterprises. Both biological and economic traits are improved with high levels of flock reproduction. Improvement of reproductive traits can have more economic impact than improving growth rate (Dickerson, 1978). Reproduction is a major contributing factor to efficiency of meat production and makes an important contribution by influencing the number of surplus animals which may be utilized for meat and contributing to current and future production through culling (Song, 2003). One of the most favorable attributes of the Black Bengal goat as a meat producing animal is its high rate of reproduction and the fact that it has an extended breeding, especially as reproduction is a major contributing factor to the efficiency of meat production.

The level of reproductive performance of goats is dependent on genetic and environmental factors, but this performance is particularly sensitive to the latter (Devendra & Burns, 1970; Riera, 1982; Song et al., 2006). Although this breed has an excellent ability to accommodate and adapt to fluctuation in environment, this often involves some degree of reproductive failure (Devendra & Burns, 1983). The goat is the most prolific of all domestic ruminants under tropical and sub-tropical conditions and certain breeds are able to breed throughout the year (Devendra & Burns, 1983), while other breeds like, for example, the Angora have a restricted breeding season (Shelton, 1978; Van der Westhuysen, 1980).

There has been a growing interest and necessity for more knowledge concerning the reproductive characteristics of farm animals, with the widespread application of artificial insemination in domestic animals. The genetic effect on each component of reproduction varies (Safari et al., 2005). Within a production or management system, the phenotypic variation of a composite trait is influenced by the level of variability among its component traits and their interactions (Snowder, 2008). Although component traits of reproduction are under the influence of many genes, a limited number of major genes associated with separate components of reproduction have been reported (Piper & Bindon, 1982; Bradford et al., 1986). Expressions of the genetic effects on reproduction are affected by numerous environmental factors such as season, climatic conditions, management, health, nutrition, breeding ratio, age and weight of doe, and libido of buck and fertility. Because genetic and environmental factors interact, genetic improvement of reproduction is very complicated (Snowder, 2007).

Flock reproductive rate also affects selection intensity and consequently the rate of genetic improvement in all traits under selection (Dickerson, 1978). Compared with improved genotype, except in terms of litter size (Wilson, 1989), the reproductive rate of small ruminants in Africa is high. However, the high reproductive potential is not usually contributing to increase production due to reproductive wastage (Mukasa-Mugerwa et al., 1992). Reproductive efficiency can be measured and expressed as the kidding rate, weaning rate, kidding interval, live weight of kids born or weaned and the length of the reproductive cycle (Greyling, 1988, 2000).

Genetic improvement in reproduction and growth traits is major goals in livestock breeding. Efficiency of livestock production is controlled by reproduction, female production and growth of offspring and concluded that improvements in reproduction were particularly likely to increase efficiency of livestock production. Development of effective genetic evaluation and improvement programmed requires knowledge of the genetic parameters for the economically important traits (Safari et al., 2005). Total weight of lamb weaned per ewe exposed to breeding provides an overall indication of ewe fertility, prolificacy, maternal performance and rearing ability as well as lamb survival and growth (Falconer & Mackay, 1996).

Reproductive efficiency is one of the important pre-conditions for increasing production potential in any given environment. In order to evaluate the productive ability of goats, prolificacy and birth weight are considered the most important and economic criteria (Morand-Fehr, 1981). The number of young born alive per kidding is an important factor in increasing productivity as it contributes more to the total weight weaned per dam than the growth rate of the kid (Bradford, 1985). It is evident that the short generation interval, non seasonality and multiple births of goat make it possible to increase production more rapidly than other ruminants in the tropics.

Reproductive merit is important consideration when evaluating the strength and weakness of new breeds in particular production environments. Breed of dam affects kid performance among various sire breeds. Reproduction fitness trait strongly influenced by environmental origins and associated selection pressures.

Thus improving the reproductive management could lead to a significant increase in productivity. The objective of the current study was (1) to evaluate the reproductive performance of Black Bengal goat and identify important factors which may influence selection of buck; and (2) to estimate variance components, heritability and breeding value for selection and ranking Black Bengal buck at different condition.

2. Materials and Methods

2.1 Location

The study was conducted in three different locations of the country under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh from April 2007 to June 2011. The regions were Tangail (Modhupur flock), Nilphamari (Dimla flock) and the Bangladesh Agricultural University (BAU) flock at the Artificial Insemination Centre.

2.2 Animals and Management

A total of 251 Black Bengal does and 22 Black Bengal bucks were used as parents for reproductive performance. Black Bengal Breeding does were reared in semi-intensive rearing system at different flock. Under the Mellonite Central Committee (MCC) and Monga Mitigation Project (MMP) selected farmers were distributed does in Madhupur flock and Dimla flock. These flocks were selected on the basis of the concentration of goat population and easiness of communication facilities and interest of the BAU and NGO workers. The farmers of each area were informed intensively for about a week for acquainting them about the various aspects and problems associated with the prevailing goat production systems and appropriate preventive measures. The objective of this joined work was to popularize AI technique and to evaluate buck effect in goat in village condition.

The animals were housed separate houses made for them or adjacent to the living rooms. Animals were grazed and supplied green grasses and concentrate feed and clinically examined regarding the health of their external genitalia. Immediate veterinary assistance was given as and when necessary. The health care package includes dipping, deworming and routine vaccination against *Peste des Petits Ruminants* (PPR). The treatment facilities were made available to the farmers on an emergency basis were advised regarding the preventive measures against different diseases. The signs of heat were observed by the farmers as well as by Field Assistants in the morning and evening. Does in heat were inseminated with frozen semen of the Black Bengal bucks maintained at BAU flock with the help of Field Assistant.

All the breeding animal and progeny were identified with neckband tags in order to maintain their individual identity and pedigree. The identities of newborns and their parents, date of insemination, date of kidding, sex of kid, litter size and parity of does were recorded. For each individual under study a record sheet with full details of each parameter along with pedigree information were maintained. New-born kids were allowed to suckle their does and were left with them up to 3-month of age. Kids were weaned at 3-month of age. Body weight (kg) was recorded in the morning before the animals were fed. Black Bengal Breeding bucks were reared at the Artificial Insemination (AI) Center under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University (BAU), Mymensingh. Bucks were reared intensively and were housed in individual pens of one square meter in galvanized iron sheet shed with a wooden slatted floor raised above the ground level. The house was provided with necessary arrangements for feeding and watering with provision of sufficient access to fresh air. The selection of buck was made on the basis of their physical character (Growth, biometry and semen characteristics) at the beginning of the study. A member of the thin family was preferred over single or triplet. Afterwards, the bucks which gave better progeny performance were kept for further breeding program and the inferior bucks determined by progeny performance were replaced by superior bucks selected on the basis of same characters described earlier. This process was continued.

The bucks were kept under zero grazing management and stall fed twice daily on a diet consisting of Napier, German and/or Maize fodder *ad libitum*. The feed was supplemented with the commercial concentrate in pellet form in the morning and again in the afternoon at the rate of 400 g/buck/day. They were allowed for exercise 1 to 2 hours daily. The breeding bucks were also supplied with germinated gram (20 g/buck/day). The weights were taken using a platform balance with an accuracy of 10 g.

2.3 Traits Analysed

The following parameters were investigated: age at 1st kidding of dams (days), weight at 1st kidding (kg) of dams, Gestation length (days) for each kidding, litter size at birth, Litter weight at birth, post-partum heat period (days), kidding interval (days).

2.4 Statistical Analyses

The significance of fixed effects (nongenetic factors) for all the traits were tested by least-squares analyses of variance using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 1998) according to the following linear model:

Statistical model for reproduction trait:

$$Yijklm = \mu + R_i + G_j + P_m + Sea_n + Eijmn$$

Where,Yijklmn is the dependent variable (individual animal record for the trait), μ is the overall mean, R_i is the effect of flock: (i=1-3), G_j is the effect of generation: (j=1-3), P_m is the effect of parity of dam: (m=1-3), Sea_n is the effect of season of birth: (n=1-3 The year was divided into three seasons; 1=winter (from November to February), 2=summer (from March to June) and 3=rainy (from July to October).

Eijmn is the residual error.

2.5 Ranking of Bucks

Deep frozen semen from 22 Black Bengal breeding bucks was used as sire line in the present study. In this study, bucks were ranked according to the predicted breeding values calculated using their progeny reproductive performance. As is known, animal breeders always try to select the best bucks for breeding with the aim that their progeny will be better than the population average.

Breeding value of animals for the traits was estimated using Best Linear Unbiased Prediction (BLUP) methodology. The BLUP in turn was carried out by computer program prediction and estimation (PEST) proposed by Groeneveld et al. (2003) using a single trait animal model.

3. Results and Discussion

Th*e* overall female reproductive traits of Black Bengal goats are provided in Table 1. The overall mean age at first kidding (days), weight at first kidding (kg), gestation length (days), litter size at birth, litter weight at birth (kg), post-partum heat (days) and kidding interval (days) were 465.6±12.45, 13.22±0.24, 145.34±0.32, 1.61±0.02, 1.66±0.04, 123.84±1.85, and 302.5±4.55, respectively. Highest coefficient of variation was observed for post-partum heat (days) (42.97%), there after for litter weight at birth (35.64%) and the lowest for gestation length (2.90%). These result was comparable with the figure reported by Hossain et al. (2004) of 401.5 days for Black Bengal goat, Ribeiro et al. (2000) of 402.28 days for Saanen goat, Kataktalware et al. (2004) of 457.71 days for Alpine x Beetal goat, Singh et al. (2004a) of 450 days for Black Bengal goat and Amit-Kumar et al. (2011) of 536.04 days for Black Bengal and Beetal x Black Bengal does. The overall weight at first kidding was observed to 13.22kg in the present study. This result was comparable with the findings reported by Hossain et al. (2004) of 15.41kg and Singh and Rai (2004b) of 15.3 kg and Chowdhury et al. (2002) of 13.50kg for Black Bengal goat. The subsequent age and weight had the similar pattern, as in first kidding.

Table 1. Overall reproductive performance of Black Bengal goats

Traits	No. of records	Least-squares means with standard error	CV (%)
Age at first kidding(days)	295	465.6±12.45	34.64
Weight at 1st kidding	269	13.22±0.24	14.65
Gestation length (days)	406	145.34±0.32	2.90
Pos-partum heat(days)	160	123.84±1.85	42.97
Kidding interval(days)	137	302.5±4.55	23.90
Litter size at birth	429	1.61±0.02	32.73
Litter weight at birth	429	1.66±0.04	35.64

The overall mean for gestation length in the present study was 145.34 days almost similar with those observed by Faruque et al. (2010) of 143.33 days and Roy et al. (2007) of 144.08 days for Black Bengal goat.

The overall mean for Pos-partum heat(days) in the present study was 123.84 days. Lower values reported by Hossain et al. (2004) of 77.29 days and Chowdhury et al. (2002) 37.0 days for Black Bengal goat and higher values reported by Singh and Roy (2003) of 213.25 days for Jamnapari goats.

The overall mean for kidding interval in the present study was 302.50 days which was almost similar with those observed by Singh et al. (2004b) of 294.92 days for Black Bengal goat, Roy et al. (2007) of 328.49 days for Saanen goats and Ribas et al. (2003) of 346.0 days for Saanen goats.

The overall litter size at birth was observed to 1.61 in the present study. This result was comparable with the findings reported by Hossain et al. (2004), Amin et al. (2001), Hoque et al. (2002) for Black Bengal goats and Hamed et al. (2009) for Zarabi goats in Egypt.

The overall litter weight at birth was observed to 1.66kg in the present study. This result was lower with the findings reported by Kataktalware et al. (2004) of 3.90kg for Alpine x Beetal goats and Laczo et al. (2006) of 3.40kg for Boer goat.

3.1 Effect of Fixed Factors

3.1.1 Age at First Kidding

The age at 1^{st} kidding in different flocks were almost similar and calculated to be 1^{st} , 2^{nd} and 3^{rd} parities (months) 14.95±0.43, 15.75±0.56, 16.25±0.95 at BAU, 16.54±0.29, 15.90±0.27, 14.96±0.60 at Madhupur 15.69±0.23, 13.63±0.42 and 15.43±0.57 at Dimla (Figure 1).

3.1.2 Weight at First Kidding

The weights of dam at first kidding were almost similar in different flocks and calculated to be 1^{st} , 2^{nd} and 3^{rd} parities (months) 10.36±0.44, 10.81±0.51, 10.25±0.84 at BAU, 9.46±0.41, 7.33±0.44, 6.35±0.64 at Madhupur and 10.84±0.14, 9.98±0.18 and 10.56±0.34 at Dimla (Figure 2).

Least-squares means and standard errors for gestation length (days), litter size at birth, litter weight at birth (kg), post-partum heat (days) and kidding interval (days) are presented in Table 2.

Table 2. Effect of different factors on the reproductive performance of goats

Factor	Litter size		Litter weight(kg)		Gestation length(days)		Post-partum heat (days)		Kidding Interval(days)	
	n	LSM± SE	n	LSM± SE	n	LSM± SE	n	LSM± SE	n	LSM± SE
Flock		*		*		NS		*		*
BAU	132	$1.53^{a}\pm0.06$	130	$2.09^{a}\pm0.04$	108	144.02±0.9	65	$120.4^{b}\pm10.4$	52	$307.8^{b}\pm15.2$
Madhupur	111	$1.45^{b}\pm0.09$	113	$1.74^{b}\pm0.06$	112	145.34±0.5	26	$130.01^{b}\pm4.9$	25	$318.6^{b}\pm18.3$
Dimla	186	$1.40^{b}\pm0.2$	186	$1.48^{b}\pm0.02$	186	147.34±0.4	60	$150.1^{a}\pm10.2$	60	$330.5^{a}\pm13.2$
Generation		*		*		NS		*		*
1	105	$1.25^{b}\pm0.05$	105	$1.80^{a}\pm0.03$	112	145.32±0.5	66	$125^{a}\pm5.1$	30	$306.15^{a}\pm15.2$
2	175	$1.58^{a}\pm0.07$	175	$2.26^{b}\pm0.05$	180	144.20±0.5	55	$115^{a}\pm7.5$	60	$295.15^{b}\pm15.2$
3	149	$1.62^{a}\pm0.06$	149	$2.40^{b}\pm0.03$	114	144.00±0.5	30	$105^{b}\pm2.45$	47	$294.00^{b}\pm15.2$
Parity		**		**		NS		*		*
1	132	$1.33^{c}\pm0.04$	130	$1.27^{c}\pm0.08$	108	144±0.53	65	$120.5^{a}\pm7.3$	52	-- - - - -
2	111	$1.54^{b}\pm0.07$	113	$1.72^{b}\pm0.13$	112	144±0.45	26	$112.21^{b}\pm5.2$	25	$240.6^{b}\pm6.3$
3	186	$1.77^{a}\pm0.09$	186	$2.21^{a}\pm0.17$	186	143±0.39	60	$90.5^{b}\pm3.8$	60	$285.9^{a}\pm10.3$
Season		NS		*		NS		*		*
Winter	132	1.54± 0.10	130	$1.23^{a}\pm0.17$	108	144.55±1.12	65	$155.3^{a}\pm12.4$	52	$288.6^{a}\pm11.3$
Summer	111	1.47±0.08	113	$1.10^{ab}\pm0.13$	112	144.80±1.12	26	$135.05^{b}\pm5.8$	25	$260.4^{b}\pm10.4$
Rainy	186	1.52±0.14	186	0.84 ± 0.12^{b}	186	143.23±1.90	60	$120.1^{b}\pm10.3$	60	$270.3^{b}\pm10.9$

Means with different superscripts within each column and trait differ significantly. NS, Not significant; *, ($P<0.05$); **, ($P<0.01$).

3.1.3 Post-Partum Heat Period

The post-partum heat period (days) varied significantly (p<0.05) within the flocks, generation, parity and season and having the lowest period for BAU flock (120.4±10.4), generation 3 (105±2.45), parity 3 (90.5±3.8), rainy season (120.1±10.3) and highest for Dimla flock (150.1±10.2), generation 1 (125±5.1), parity 1 (120.5±7.3) and winter season (155.3±12.4) (Table 2).

3.1.4 Kidding Interval

The period between two consecutive kiddings were analysed for each individual animal. The result of least-squares analysis showed that the flocks, generation, parity and season for kidding interval are significant (p<0.05) and had the similar trend as in post-partum heat period (days) having the lowest value for BAU flock (307.8±15.2), for generation 3 (294.00±15.2). The lowest value for kidding interval was at parity 2 (240.6±6.3) and summer season (260.4±10.4). The highest value for kidding interval was at Dimla flock (330.5±13.2), generation 1 (306.15±15.2), parity 1 (285.90±10.3) and winter season (288.6±11.3) (Table 4.13). The pos-partum heat (days) and kidding interval (days) was found (Table 2) significant (P<0.05) in this study. In case of kidding interval same result was found by (Wilson et al., 1989), which ranged from 238-265days. Higher value 349.41 to 362 has reported by (Lobo et al., 2005) than this study. The increase in productivity with parity indicates improvement of reproductive traits as does reach maturity.

3.1.5 Litter Size and Litter Weight

Litter size and litter weight were analysed from the individual kidding records for each animal in different flocks, generations, parities and seasons (Table 2). The analysis of variance for litter size and litter weight at different flocks (p<0.05), generation (p<0.05), parity (p<0.01) are significant. The least-squares analyses of variance for liter size and litter weight are presented in Table 2. The highest litter size and litter weight were observed at BAU flock (1.53±0.06 and 2.09±0.04 kg) and lowest at Dimla flock (1.40±0.2 and 1.48±0.02 kg). Trends of increasing the litter size and litter weight are observed with the advancement of generation and parities (Table 2). Similar results was reported by Faruque et al. (2010) for litter size at birth of (1.33 to 1.77) and litter weight at birth (1.27 to 2.21kg) increased significantly (P<0.01) as parity progressed. Hussain et al. (2004) noticed that among the four parities, kid's birth weight was lowest in 1st parity does compared to 2nd, 3rd and 4th parity. This may be due to improved efficiency of reproduction as the doe matures. The increase in litter size was reported to continue to the fourth parity and six (Fogarty et al., 2000) years of age and declined thereafter. The increase in litter size with advance in age and parity is the result of increased ovulation rate, uterine capacity and other maternal traits affecting the reproductive efficiency (Fahmy, 1990). Parity of doe significantly influenced litter size at birth, litter weight (kg) at birth and, first kidding being the smallest which was in agreement with the findings of Chowdhury et al. (2002), Marai et al. (2002), Hoque et al. (2002), Song (2003) and Hamed et al. (2009).

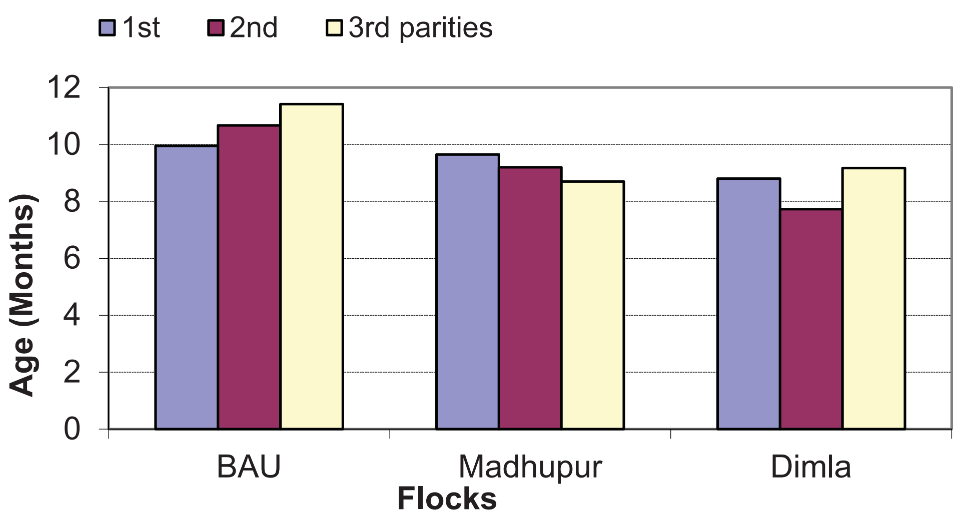

Figure 1. Age of dams in different partities

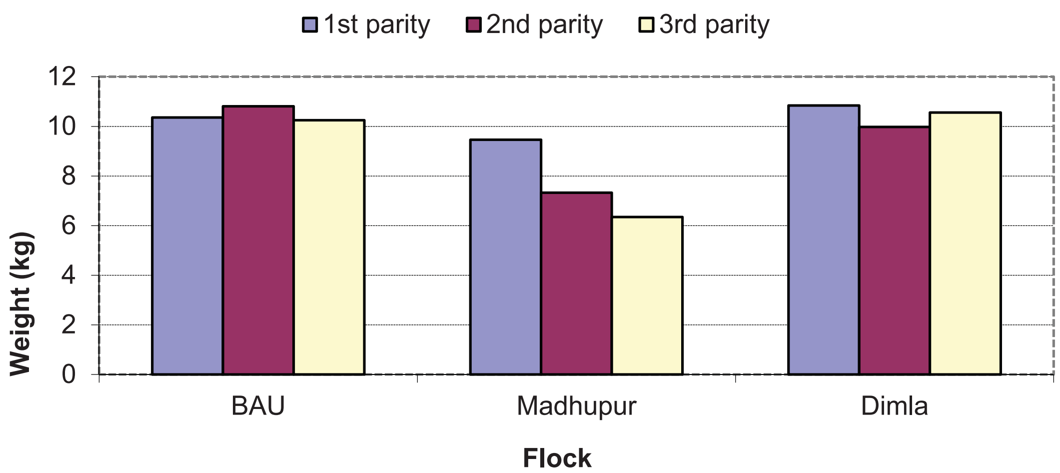

Figure 2. Weight of dams in different parities

3.1.6 Gestation Length

Gestation length of each goat in different flock, generation and parities was analysed. The lowest gestation length was obtained at flock BAU (144.02±0.9 days), at generation 2 and 3 (144.20±0.5 days) at 3rd parity 143±0.39 days and at rainy season 143.23±1.90 days (Figure 4.11). No significant difference was found for this trait (Table 4.13). For gestation length flock effect and generation effect was not significant. Similar results was reported by Faruque et al. (2010) for generation one143.00 days and generation two 141.25 days. The effect of parity on gestation length was found to be non-significant in the present study. Almost similar findings for gestation length were reported by Chowdhury et al. (2002), Kataktalware et al. (2004) and Hossain et al. (2004), Roy et al. (2007), Chun-Yan Zhang et al. (2009), in different goats. The factors which are responsible for the variation of gestation length and kidding interval may be equally considered for the variation of post-partum heat in different regions, parities and seasons (Husain, 2000).

The present study indicated that season of kidding had no significant influence on the Litter size and gestation length. Litter weight, post-partum heat (days) and kidding interval in Black Bengal goats were significant ($P<0.05$) in the present study which was supported by Browning et al. (2011) and Gbangboche et al. (2006) and Hamed et al. (2009). It is observed that there is minimal rainfall during the winter which intern responsible for green grasses on the field, is only the available means for feeding the goats. This period is mainly covered with the late part of winter and early part of summer and is considered as the most critical time for grazing the animals on the field. Tree leaves are also very limited for growth during this period.

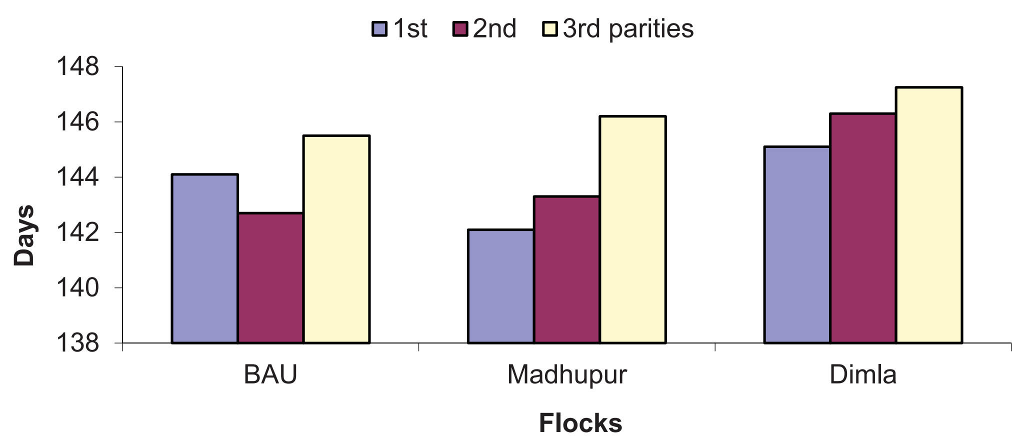

Figure 3. Gestation lengths of does in different parities

3.2 Variance Components and Heritability

Estimates of additive genetic variance (σ^2a), residual variance (σ^2e), phenotypic variance (σ^2p) and heritability (h^2) of age at first kidding, weight at first kidding, litter size at birth, litter weight at birth, gestation length, post-partum heat and kidding interval and repeatability of litter size at birth and kidding interval of Black Bengal kids are in Table 4.14 which are comparable to those results reported by Ribeiro et al. (2000), Lobo et al. (2005), Menendez et al. (2003), Rao and Notter (2000), Neopane (2000), Ricordeau (1991), Bagnicka (2007), Sarmento et al. (2003) and Vatankhah et al. (2008). It appears from the table that heritability estimates of those traits are rather low, and reflect the generally small genetic variance for the reproductive traits. Low heritability of reproductive traits is probably due to greater proportional influence of environmental effects as well as little genetic variability for fertility, litter size and other reproductive traits.

Table 3. Estimates of additive genetic variance (σ^2a), residual variance (σ^2e), phenotypic variance (σ^2p) and heritability (h^2) of age at first kidding, weight at first kidding, litter size at birth, litter weight at birth, gestation length, post-partum heat and kidding interval and repeatability of litter size at birth and kidding interval of Black Bengal kids

Traits	σ^2a	σ^2e	σ^2p	h^2	r_p
Age at first kidding	1.383	5.206	6.589	0.21±0.11	
Weight at first kidding	1.53	6.991	8.521	0.18±0.10	
Litter size at birth	0.026	0.159	0.185	0.14±0.12	0.16±0.12
Litter weight at birth	0.045	0.331	0.376	0.12±0.08	
Gestation length	1.668	5.191	7.582	0.22±0.11	
Post-partum heat	0.876	2.778	3.654	0.24±0.14	
Kidding interval	0.974	4.761	5.735	0.17±0.11	0.045±0.04

3.3 Predicted Breeding Value and Ranking of Bucks

Breeding values as calculated by BLUP technique for reproductive performance were ranked then according to their genetic worth. On the basis of PBV of litter size at birth, litter weight at birth, age at first kidding, weight at first kidding, gestation length, post-partum heat period and kidding interval were estimated and according to value prioritized bucks were selected (Figure 4). Column of the entire figure show the predicted breeding value for each individual. The highest breeding value of reproductive traits was found in the progeny of Buck No. 32, followed by Buck No. 52, 54, 81 and 87. The lowest breeding value of reproductive traits was found from the progeny of Buck No. 3 followed by Buck No. 11 and 4 (Figure 4).

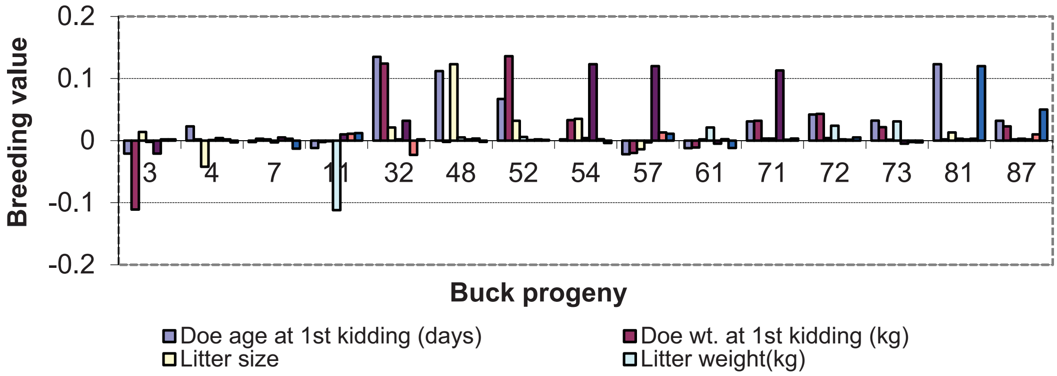

Figure 4. Predicted breeding value of different reproductive traits of different bucks' progeny

4. Conclusion

Estimates of heritability for reproductive traits in this study were quite low and hence presence of large environmental variances, indicating that possibility of selection based on doe's own performance to improve these reproductive traits would take a long time and improvement in these traits through selection may be limited. So, Bucks may be selected through progeny testing maintaining the appropriate reproductive management program addressing most important individual traits which are directly involved for increasing lifetime productivity.

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Dry Matter Production and Harvest Index of Groundnut (*Arachis hypogaea L.*) Varieties Under Irrigation

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Abstract

Dry matter production for crops is generally influenced by the fertility status of the soil. Plant population may indirectly affect the amount of dry matter production due to its relationship with number of plants per unit area. An experiment to study the effect of plant population and basin size on dry matter production and other yield components of groundnut (*Arachis hypogaea* L.) varieties under irrigation was conducted during the dry seasons of 2004 to 2006 at the Irrigation Research Station of the Institute for Agricultural Research (IAR), Ahmadu Bello University at Kadawa. The treatments tested were three basin sizes (3m x 3m, 3m x 4m and 3m x 5m), three plant populations (50,000, 100,000 and 200,000 plants ha^{-1}and three varieties (SAMNUT 23, SAMNUT 21 and SAMNUT 11). These were arranged factorally in a split plot design with plant population and variety assigned to the main plots and basin sizes in the sub plots. SAMNUT 23 had higher harvest index than the other varieties, however SAMNUT 11 recorded the highest dry matter plant^{-1}. Dry matter production was significantly highest at 100.000 plants ha^{-1}, while significantly highest harvest index was observed at 200,000 plants ha^{-1}. SAMNUT 23 exhibited highest harvest index compared to the other varieties used in this study.

Keywords: groundnut varieties, irrigation, dry matter production, plant spacing

1. Introduction

Groundnut is an annual legume cultivated throughout the tropics and beyond; its cultivation is limited by the occurrence of frost (Anonymous, 2000). It is a short herbaceous annual belonging to the subfamily *papilionaceae* of the family *Leguminosae*. Essentially groundnut is a warm season crop requiring abundant sunshine for normal development. The crop development and maturity period are mainly dependent on the temperature (De Waele and Swanevelder, 2001). The mean daily temperature for optimum growth is 22 to 28°C; a reduction in yield occurs above 33°C and below 18°C (Anonymous, 2002). It also requires enough soil moisture from the beginning of flowering up to two weeks before harvest. Dry matter production and accumulation in most arable crops is affected by photosynthesis and respiration. These factors which ultimately influence crop performance are affected by climate, soil fertility, moisture availability, pests and diseases, as well as socio-cultural practices. Amongst the cultural practices, plant spacing and density have significant effect on dry matter production.

Bell et al. (1987) observed that biological yield (above ground biomass plus pods) increased markedly (12,600-16,900 kg ha^{-1}) with increasing density up to the maximum of 588,000 plants ha^{-1}. Especially in the Sudan savanna regions, groundnut haulms serve as excellent source of feed for livestock as well as organic matter source for soil improvement. The groundnut crop may be grown for both the seeds and for fodder. To this end dual purpose groundnut varieties have been bred at IAR Zaria providing farmers with both seed and fodder for human consumption and for livestock res [ectively. Similarly there are varieties bred for high seed yield although moderate amounts of fodder may be obtained: thus there is a need to explore ways to increase output of this important feed source for livestock.

Harvest index (HI), a measure of crop yield is the weight of harvested product as a percentage of the total plant weight of a crop. The concept has been used in crop improvement and physiology. Ahmad et al. (2007) observed that low crop harvest index is the major cause of less crop yield. They further opined that low crop HI could be attributed to cultivation of non recommended crop cultivars, unapproved seed for sowing, late sowing, imperfect sowing methods, low plant population, poor plant protection and proliferation of weeds, unbalanced use of fertilizer and non availability of water for irrigation at critical growth stages. However, studies by Bindi et al. (1999) show that change in harvest index is stable over a range of growth conditions which include irrigation and fertility treatments. In an experiment with four groundnut genotypes, Bell et al. (1992) found that change in harvest index over time varied from 0.0050 to 0.0140 HI d^{-1}.

Conventionally, groundnut cultivation in Nigeria is carried out mostly by smallholder farmers during the wet season under rain fed conditions with limited inputs. However where rainfall is not adequate the crop can be grown under irrigation.

Groundnut production with irrigation is an uncommon practice in the Sudan savanna zone of Nigeria. Results of research conducted elsewhere in the world however have shown that pod and haulm yields of groundnut obtained under irrigation are higher than those obtained during the rainy season. Studies by Ishag (2000) on the growth of groundnut with irrigation have shown that the provision of adequate moisture during important phenological growth stages has a marked influence in terms of dry matter production, economic yield and yield components. This is due to the fact that there is better control over moisture supply during an irrigated than a rainfed season. Moreover mid season, end of season and other inconsistensis associated with with rainy season are avoided under irrigated conditions. This experiment was thus conducted to study the dry matter production and harvest index of groundnut varieties at varying plant populations under irrigation.

2. Materials and Method

2.1 Experimental Site

Field trials were conducted at the Irrigation Research Substation of the Institute for Agricultural Research, Ahmadu Bello University Kadawa (11°39'N, 08°27'E; altitude 500 m) during the 2003/2004, 2004/2005 and 2005/2006 dry seasons. The substation is located in the Sudan Savanna ecological zone of Nigeria where the dry season starts in October to the end of May.

2.2 Land Preparation

After harrowing and ridging at 0.75 m width, the field was marked out into plots and thereafter leveled. The gross plots were 3 m x 3 m; 3 m x 4 m and 3 m x 5 m of about 20 cm depth. Sowing was done at a fixed inter-row plant spacing of 50 cm. The intra-row plant spacing was however varied at 40, 20 and 10 cm leading to plant density of 50000, 100000 and 200000 plants ha^{-1} respectively.

2.3 Sowing

In this process 2 seeds per hole were sown at a depth of about 3 cm by hand in all the years of the experiment.

The crop was fertilized with 20 kg N, 23.6 kg P and 24.9 K/ha^{-1} as basal dose using urea, single super phosphate (SSP) and muriate of potash (MOP)

2.4 Weed Control

This was done by pre-emergence application of Metolachlor + Terbutryne (Igram Combi$^{(R)}$ 500EC) at the rate of 4 liters ha^{-1} with a CP20 knapsack sprayer. Hoe weeding at four (4) and eight (8) weeks after sowing (WAS) was done in order to remove the weeds that emerged later.

2.5 Harvesting

The net plots were harvested by digging out the whole plant including the pods with a hoe and picking up the remaining pods from the soil.

2.6 Data Collection

Starting from 3WAS two plants were randomly selected from the border rows in each gross plot, at 3, 6, 9 12 WAS and at harvest, uprooted and oven dried to constant weight for 48 hours at 70°C. The oven-dried samples were weighed using a Mettler balance model P1200 and the value recorded to obtain the total dry matter. Harvest index: the ratio of the seed yield to the total dry matter was calculated at harvest using the formula:

$$K = \frac{\text{Grain yield from sample of TDM}}{\text{X g TDM sample}} \times 100$$

Where K = harvest index, TDM = Total dry matter (g).

2.7 Data Analysis

Data collected were subjected to statistical analysis of variance using SAS (SAS Institute Inc.) statistical software. Where the F values were found to be significant, the treatment means were separated using Duncan Multiple Range Test DMRT (Duncan, 1955).

3. Results

At 3 WAS, the effect of plant population on total dry matter (TDM) was significant only in 2004/2005 and 2005/2006 and when the result was combined (Table 1). TDM was significantly higher at 200,000 plants ha^{-1} in 2004/2005, 2005/2006 and the combined but was statistically at par with that at 100,000 plants ha^{-1}. The least TDM was produced at 50,000 plants ha^{-1}. The combined result showed that significantly highest TDM was achieved at 200,000 and 100,000 plants ha^{-1}. The varieties exhibited significant differences in their TDM across the years. SAMNUT 11 produced significantly higher TDM than SAMNUT 21 in 2003/2004 but was statistically at par with TDM of SAMNUT 23. In 2004/2005, SAMNUT 21 had significantly higher TDM than SAMNUT 23 and 11. When the result was combined, TDM from, SAMNUT 21 was higher than SAMNUT 23, but was statistically at par with SAMNUT 11.

Table 1. Effect of plant population and basin size on total dry matter (g) at 3WAS of three groundnut varieties during 2003/2004, 2004/2005 and 2005/2006 dry season at Kadawa

Treatments/Years	2003/2004	2004/2005	2005/2006	Combined
Plant Population (‘000 plants ha^{-1})				
50	0.98	1.56b	1.11b	1.22b
100	1.05	1.75ab	1.24ab	1.35a
200	1.16	1.95a	1.39a	1.50a
SE ±	0.052	0.122	0.064	0.110
Variety				
SAMNUT 23	1.05ab	1.61b	1.16	1.27b
SAMNUT 21	0.92b	2.05a	1.32	1.43a
SAMNUT 11	1.21a	1.61b	1.25	1.36ab
SE ±	0.052	0.122	0.064	0.110
Basin size				
3m x 3m	1.07	1.69	1.22	1.33
3m x 4m	1.05	1.89	1.31	1.42
3m x 5m	1.06	1.68	1.20	1.31
SE ±	0.063	0.097	0.061	0.074
Interaction				
P x V	NS	NS	NS	NS
P x B	NS	NS	NS	NS
V x B	NS	NS	NS	NS
P x V x B	NS	NS	NS	NS

NS- Not significant. Means followed by the same letter within the same treatment group and year are statistically the same.

At 6 WAS there were no significant changes in TDM as a result of changes in plant population (Table 2). The varieties were not significantly different in their TDM in 2004/2005, 2005/2006 and combined. However in 2003/2004, SAMNUT 23 and SAMNUT 11 had significantly higher TDM than SAMNUT 21.

Table 2. Effect of plant population and basin size on total dry matter (g) at 6WAS of three groundnut varieties during 2003/2004, 2004/2005 and 2005/2006 dry season at Kadawa

Treatments/Years	2003/2004	2004/2005	2005/2006	Combined
Plant Population (‘000 plants ha^{-1})				
50	1.71	6.47	2.99	3.72
100	1.77	6.47	3.04	3.76
200	1.65	6.89	3.19	3.91
SE ±	0.080	0.483	0.246	0.427
Variety				
SAMNUT 23	1.98a	6.34	2.94	3.77
SAMNUT 21	1.26b	7.33	3.13	3.91
SAMNUT 11	1.89a	6.31	2.93	3.71
SE ±	0.080	0.483	0.246	0.427
Basin size				
3m x 3m	1.67	6.42	2.92	3.70
3m x 4m	1.80	6.76	3.12	3.89
3m x 5m	1.66	6.71	3.02	3.80
SE ±	0.081	0.431	0.222	0.257
Interaction				
P x V	NS	NS	NS	NS
P x B	NS	NS	NS	NS
V x B	NS	NS	NS	NS
P x V x B	NS	NS	NS	NS

NS- Not significant. Means followed by the same letter within the same treatment group and year are statistically the same.

At 9 WAS there were no significant differences in TDM due to plant population in 2003/2004 (Table 3). In 2004/2005 and 2005/2006 however, TDM was significantly higher in the 200,000 plants ha^{-1} than the 50,000 plants ha^{-1} but was statistically at par with the 100,000 plants ha^{-1}. The combined result showed that a significantly higher TDM was attained at the 200,000 plants ha^{-1} and 100,000 plants ha^{-1} than at the 50,000 plants ha^{-1}. There were significant differences among the varieties in their TDM in the three years. SAMNUT 23 and SAMNUT 11 produced significantly higher TDM than SAMNUT 21 in all the years and combined although SAMNUT 23 was at par with SAMNUT 21 in 2004/2005. There were significant interactions ($P \leq 0.05$) between plant population and variety in 2003/2004; and plant population and basin size in the combined. In 2003/2004 season the variety by population interaction showed that significantly highest total dry matter was produced by SAMNUT 11 at 50,000 plants ha^{-1}. This was statistically similar to that produced by SAMNUT 23 at 100,000 plants ha^{-1} (Table 4). SAMNUT 21 produced its highest TDM at 200,000 plants ha^{-1}. For 3m x 3m and 3m x 4m each increase in plant population resulted to an increase in TDM. The widest basin size having the highest plant population gave the highest TDM plant^{-1}. Significantly highest total dry matter was obtained at 200,000 plants ha^{-1} in the 3m x 5m basin, the least being observed in the 3m x 3m basin at 50,000 plants ha^{-1} (Table 4). For the 3m x 5m basin increase in plant population to 100,000 plants ha^{-1} led to significant reduction in TDM, however further increase in plant population to 200,000 plants ha^{-1} in this basin led to significant increase in TDM.

TDM at 12WAS was unaffected by the different plant population (Table 5) in all the years and when the results were combined. Significant differences were observed between varieties in their TDM production in all the years and when combined. SAMNUT 11 produced the highest TDM at 12 WAS in all the years and when combined but was similar to SAMNUT 21 in 2005/2006. SAMNUT 21 had significantly higher TDM than SAMNUT 23 in 2005/2006 and the combined but was similar to SAMNUT 23 in 2003/2004 and 2004/2005.

Table 3. Effect of plant population and basin size on total dry matter (g) at 9WAS of three groundnut varieties during 2004, 2005 and 2006 dry season at Kadawa

Treatments/Years	2004	2005	2006	Combined
Plant Population (‘000plants ha^{-1})				
50	3.60	25.52b	13.39b	14.17b
100	3.82	31.31ab	16.34ab	17.12a
200	3.39	34.85a	17.45a	18.23a
SE ±	0.200	2.082	1.092	1.891
Variety				
SAMNUT 23	4.36a	31.43ab	16.72a	17.50a
SAMNUT 21	2.57b	26.11b	13.17b	13.95b
SAMNUT 11	3.88a	33.30a	17.29a	18.07a
SE ±	0.200	2.082	1.092	1.891
Basin size				
3m x 3m	4.14a	26.24b	13.99	14.79a
3m x 4m	3.42b	31.26ab	16.17	16.95ab
3m x 5m	3.25b	33.12a	16.99	17.78a
SE ±	0.216	2.225	1.114	1.378
Interaction				
P x V	*	NS	NS	NS
P x B	NS	NS	NS	*
V x B	NS	NS	NS	NS
P x V x B	NS	NS	NS	NS

* Significant at 5 percent level. NS- Not significant. Means followed by the same letter within the same treatment group and year are statistically the same.

Table 4. Interaction between basin size and plant population and variety and plant population at 9WAS on TDM $plant^{-1}$ for the combined and 2003/2004 season

	2003/2004 Plant Population		
	50	100	200
SAMNUT 23	4.24b	4.68a	4.16b
SAMNUT 21	1.87f	2.55e	3.27c
SAMNUT 11	4.70a	3.27c	2.73d
SE± 0.375			
3m x 3m	10.70d	16.70b	16.96b
3m x 4m	13.56c	18.99ab	18.32ab
3m x 5m	18.26ab	15.67c	19.42a
SE± 2.38			

Means followed by the same letter(s) within the same row or columns are statistically similar at P≤ 0.05.

Table 5. Effect of plant population and basin size on total dry matter (g) at 12WAS of three groundnut varieties during 2003/2004, 2004/2005 and 2005/2006 dry season at Kadawa

Treatments/Years	2003/2004	2004/2005	2005/2006	Combined
Plant Population (‘000plants ha^{-1})				
50	51.18	143.14	94.23	96.18
100	55.92	141.65	95.86	97.81
200	50.59	149.21	96.97	98.92
SE ±	3.136	9.984	5.283	9.150
Variety				
SAMNUT 23	47.70b	121.13b	79.94b	82.92c
SAMNUT 21	54.33b	145.50ab	95.44a	98.42b
SAMNUT 11	61.66a	164.48a	108.60a	111.57a
SE ±	3.136	9.984	5.283	9.150
Basin size				
3m x 3m	52.11	144.43	93.84	96.79
3m x 4m	57.29	146.11	97.27	100.22
3m x 5m	54.29	140.86	92.59	95.90
SE ±	2.339	8.501	4.642	5.327
Interaction				
P x V	NS	NS	NS	NS
P x B	*	NS	NS	NS
V x B	NS	NS	NS	NS
P x V x B	NS	NS	NS	NS

* Significant at 5 percent level. NS- Not significant. Means followed by the same letter within the same treatment group and year are statistically the same.

Table 6. Interaction between basin size and plant population at 12WAS for TDM plant^{-1} for the 2003/2004 season

	Basin Size		
	3m x 3m	3m x 4m	3m x 5m
Plant Population (‘000plants ha^{-1})			
50,000	11.47f	18.73b	13.61e
100,000	19.15b	18.07bc	14.65e
200,000	20.83a	17.32cd	14.30e
SE± 1.718			

Means followed by the same letter(s) within the same row or column are statistically similar at P≤ 0.05.

A significant interaction was observed between plant population and basin size in 2003/2004 and this is shown in Table 6. The smallest basin at the highest plant population produced the highest TDM. For 3m x 3m and 3m x 4m basins the higher the plant population the more TDM realized. For the 3m x 5m however, no significant difference was observed as plant population increased from 50,000 to 200,000 plants ha^{-1}. At 50,000 plants ha^{-1} increasing the basin size to 3 m x 4 m led to significantly more TDM; further increase to 3m x 5m led to significantly low TDM. At 100,000 and 200,000 plants ha^{-1} increase in basin size to 3 m x 5 m progressively led to reduction in total dry matter.

At harvest, plant population only in 2004/2005 significantly affected TDM when the 100,000 plants ha^{-1} resulted in more TDM than the 50,000 plants ha^{-1} and 200,000 plants ha^{-1} (Table 7). In other years and when combined, no significant differences were observed. In 2004/2005 significant differences among varieties in their TDM were observed. SAMNUT 21 and SAMNUT 11 had more TDM than SAMNUT 23 but SAMNUT 11 was at par with SAMNUT 23. In other years and the combined no significant differences were observed.

Table 7. Effect of plant population and basin size on total dry matter (g) at harvest of three groundnut varieties during 2003/2004, 2004/2005 and 2005/2006 dry season at Kadawa

Treatments/Years	2003/2004	2004/2005	2005/2006	Combined
Plant Population (‘000plants ha^{-1})				
50	214.46	177.46b	296.64	229.52
100	218.04	227.31a	296.28	247.21
200	196.73	174.55b	302.21	224.50
SE ±	14.569	16.915	39.390	27.177
Variety				
SAMNUT 23	209.14	164.75b	294.70	222.86
SAMNUT 21	212.91	220.45a	297.62	243.73
SAMNUT 11	207.18	194.12ab	302.62	234.64
SE ±	14.569	16.915	39.390	27.177
Basin size				
3m x 3m	220.44	181.92	291.43	231.27
3m x 4m	213.81	219.00	318.75	250.52
3m x 5m	194.97	178.39	284.95	219.44
SE ±	10.421	14.722	20.193	18.505
Interaction				
P x V	NS	NS	NS	NS
P x B	NS	NS	NS	NS
V x B	NS	NS	NS	NS
P x V x B	NS	NS	NS	NS

*Significant at 5 percent level. NS- Not significant. Means followed by the same letter within the same treatment group and year are statistically the same.

Table 8. Effect of plant population and basin size on harvest index of three groundnut varieties in 2003/2004, 2004/2005 and 2005/2006 dry season at Kadawa

Treatments/Years	2003/2004	2004/2005	2005/2006	Combined
Plant population (‘000plants ha^{-1})				
50	31.94b	18.57b	25.16	25.22b
100	36.13b	14.37c	24.80	25.10b
200	39.70a	22.02a	27.10	29.78a
SE ±	2.618	1.546	2.443	2.469
Variety				
SAMNUT 23	48.30a	25.55a	31.69a	35.18a
SAMNUT 21	26.59b	13.12c	19.90c	19.87c
SAMNUT 11	32.88b	16.29b	25.98b	25.05b
SE ±	2.618	1.546	2.443	2.469
Basin size				
3m x 3m	38.61	18.87	24.06	27.18
3m x 4m	35.69	18.47	27.43	27.19
3m x 5m	33.48	17.61	26.08	25.72
SE ±	2.455	0.994	1.593	1.922
Interaction				
P x V	NS	NS	NS	NS
P x B	NS	NS	NS	NS
V x B	NS	NS	NS	NS
P x V x B	NS	NS	NS	NS

NS- Not significant. Means followed by the same letter within the same treatment group and year are statistically the same.

The effect of plant population on harvest index (HI) was significant in 2003/2004 and 2004/2005 (Table 8). In 2005/2006, no significant effect was observed. The combined result revealed that HI was significantly highest when groundnut was planted at 200,000 plants ha^{-1}. HI of the varieties was significantly different. SAMNUT 23 exhibited the highest HI compared to SAMNUT 21 and SAMNUT 11 across the years and when over three years averaged. Basin size had no significant effect on HI in all the years and the combined.

4. Discussion

The results reveal that plant population and basin size have variable effects on dry matter production and yield of groundnut. These effects differed according to variety and growing season. Initially increase in plant population from 50,000 to 200, 000 plants ha^{-1} led to increase in TDM, however this was evident only during vegetative growth stages and early reproductive stages. When the crop attained full canopy closure the difference in TDM was slight. This means that increasing the plant population led to increase in TDM until an optimum was reached beyond which further increases in number of plants did not produce significant changes in TDM. The results show that more dry matter was produced at the higher plant population. This is attributed to the fact that due to higher competition between plants individual dry matter decreased significantly compared to high total mass from a community of plants because of the higher number of plants per unit area. In experiments with Napier grass, Wijitphan et al. (2009) reported a significant effect of plant spacing on dry matter production at 50x40.

Similar results were reported by Tanimu et al. (1998) with different plant spacing. This result is similar to the work of Nedelcu et al. (1986) who observed maximum dry matter accumulation of 62 g/plant at 100,000 plants ha^{-1}. On the hand Bell et al. (1987) observed an increase in biological yield with increasing plant population from 88,000 to 394,000 plants ha^{-1}. Crops sown at high density are able to attain faster canopy cover than those at low density by especially by harnessing solar radiation, nutrients and moisture. The increased dry matter accumulation is related to the lesser time required for plants in high density plant spacing's to achieve a higher solar radiation interception than plants in the lower plant population. Thus total dry matter increases until a point is reached where increases in plant numbers has no positive impact on the total dry matter achievable.The low dry matter recorded at 200,000 plants ha^{-1} is also an indication of competition for available moisture. Although in this study water was not a limiting due to availability of irrigation water, the higher numbers of plants (200000 plants ha^{-1}) per unit area could have led to a higher than normal moisture demand than was obtained in plots having 100000plants ha^{-1} or 50000 plants ha^{-1}. Moisture stress is known to have adverse effects on yield. Drought stress at 50% flowering in chick peas was found to have a more damaging effect on dry matter production than drought stress at 50% podding phase (Patel & Hemantaranjan, 2012). (Tatar & Gevrek, 2008) observed a decline in dry matter produced by wheat after onset of stress treatment. Research by Travlos and Karamanos (2008) revealed the beneficial effect of adequate water supply on growth and dry matter production of marama.

Looking at the dry matter produced per plant across the three growing seasons, slightly warmer temperatures during 2005 and 2006 resulted to more vigorous plant growth. The differences in dry matter produced during the years of experimentation are attributed to seasonal variations in temperature (data not shown). Anonymous (2002) reported that cultivation of groundnut is limited by the occurrence of frost. On the other hand extremely high temperatures are detrimental to growth and development of the crop (Ishag, 2000). Consequently total dry matter was generally lower in 2003/2004 than in 2004/2005 and 2005/2006. The slower growth rate due to the cooler conditions during early stages caused slower biomass production and accumulation in successive stages indicating the importance of initial crop growth for final yield.

Vara Prasad et al. (2000) also found that exposure to high air and or soil temperatures significantly reduced dry matter production, partitioning of dry matter to pods and pod yields. Irrespective of season the results reveal an increasing trend in dry matter over the sampling period.

A differential response of the varieties was attributed to variation in their genetic makeup. Throughout the early growth stages (3 and 6 WAS) differences in dry matter production among the varieties were slight. This pattern was similar for the three years. However at 9 and 12 WAS SAMNUT 11 displayed superiority in terms of dry matter production. The trend was reversed by harvest time when SAMNUT 21 exhibits the highest dry matter production. Although SAMNUT 21 and SAMNUT 11 produced significantly higher amount of dry matter than SAMNUT 23, which is expected, SAMNUT 21 produced the highest total dry matter plant^{-1}, while SAMNUT 11 produced highest total dry matter hectare^{-1}. The high dry matter yield observed in SAMNUT 11 is attributed to its more profuse branching habit. This was due to its superiority in growth attributes such as number of branches per plant and number of leaves per plant (data not shown). As a late maturing crop, which completes its life cycle in 130-150, more time was allocated to partitioning of assimilates to vegetative parts, which include leaves, stem and branches in addition to other reproductive parts. There is an indication that there are differences in partitioning of dry matter to pod by the different varieties in response to environmental factors such as temperature. However dry matter accumulation in each part of the plants continued until maturity although production rate differed depending on variety and age.

Harvest index; the ratio of biomass yield to pod yield is an important indicator of yield in groundnut. There was very little variation in the harvest index indicating high stability of this character in the groundnut crop. SAMNUT 23 exhibited highest harvest index under irrigated conditions. Muldoon (1985) reported that differences between cultivars were related to differences in harvest index and in the rate of pod filling.

In conclusion, the study has revealed that dry matter production for a community of crops increases with increase in plant population until a point is reached where increases in population do not lead to corresponding significant increases in production of dry matter. For our study, highest dry matter production was observed at 100.000 plants ha^{-1}, while harvest index was significantly highest at 200,000 plants ha^{-1}. Of the varieties used, SAMNUT 23 exhibited highest harvest index.

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Effect of Agricultural Extension Program on Smallholders' Farm Productivity: Evidence from Three Peasant Associations in the Highlands of Ethiopia

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Abstract

This study evaluates the effect of agricultural extension program participation on farm productivity taking three case study *kebeles* (peasant associations) in Ethiopia. A total of 1112 plot-level data collected from 300 selected farm households, comprising of extension participants and non-participants, were used in the study. The study begins the estimation with simple Ordinary Least Square (OLS) method. To deal with the potential bias due to the existence of observed and unobserved characteristics, we employed Heckman Treatment Effect Model (HTEM) and Propensity Score Matching (PSM). The OLS result shows that extension participation increases farm productivity by about 6%. However, both HTEM and PSM clearly reveal the presence of selection bias in extension program participation which leads to underestimation of the OLS estimates. The participation could have increased farm productivity by up to 20% had it not been to the serious selection bias related to non-farming factors such as involvement in *kebele* administration, and wealth status of the participants observed during program placement. Our PSM analysis also verifies the positive effect of extension program participation on farm productivity. In conclusion, the extension program has a positive effect on farm productivity in the study area. However, its effect with its current structure and input could have tripled had there been no bias related to extension program participation. This result provides a valuable policy insight in which improving access to diversified and quality agricultural inputs are critically necessary for the participants on top of expanding the program to less resourceful farmers by avoiding any entry barriers in the future.

Keywords: extension program participation, farm productivity, selection bias, Ethiopia

1. Introduction

Increasing agricultural productivity is a major challenge in Sub-Saharan Africa (SSA), where 62% of the population (excluding South Africa) depends on agriculture for their livelihoods (Staatz & Dembele, 2007). Since 1960s, agricultural production in SSA has failed to keep up pace with population growth (Benin, 2006). Improving the productivity, profitability, and sustainability of smallholders farming is therefore the main pathway to get out of poverty (World Development Report [WDR], 2008). It is widely argued that, achieving agricultural productivity growth will not be possible without developing and disseminating improved agricultural technologies that can increase productivity to smallholder agriculture (Asfaw, Shiferaw, Simtowe, & Lipper, 2012).

Like in many other SSA countries, agriculture is the most important sector for sustaining growth and reducing poverty in Ethiopia. It accounts for 85% of employment, 50% of exports, and 43% of gross domestic product (GDP) (FAO, 2010). However, lack of adequate farm management practices, low level of modern inputs usage, the depletion of soil organic matter and soil erosion, highly rain fed dependent agriculture system are major obstacles to sustain the agricultural production in the country (Grepperud, 1996; Pender & Gebremedhin, 2007; Kassie, Zikhali, Manjur, & Edward, 2009).

In cognizant of these problems, the government of Ethiopia launched a strategy which is known as the Agricultural Development Led Industrialization (ADLI) in 1993 that sets out agriculture as a primary stimulus to generate increased output, employment and income for the people, and as the spring board for the development of the other sectors of the economy (Kassa & Abebaw, 2004; Gebremedhin, Jalata, & Hoekstra, 2009). One of the major components of ADLI is the national extension package program known as Participatory Demonstration and Training Extension System (PADETES). The objective of PADETES are to achieve sustainable development in rural areas through increasing farm productivity (yield), reducing poverty, increasing the level of food security, increasing the volume and variety of industrial raw materials (primary products), and producing for the export market (Kassa, 2003; Ethiopian Economics Association [EEA], 2006).

The PADETES program has been focusing on supply-driven intensification which consists of enhanced supply and promotion of improved seeds, fertilizers, on-farm demonstrations of improved farm practices and technologies and close follow up of farmers' plots (Kassa & Abebaw, 2004; EEA, 2006; Kassa, 2008). Hence, wider dissemination of improved farm technologies, management practices and know-how to the smallholder farmers have been the major activities of the extension program (Kassa, 2003; Gebremedhin et al., 2009; Asfaw et al., 2012).

However, the performance of the agriculture sector has been very dismal in spite of implementing the national extension package program-PADETES. The impacts of the implemented technologies have been mixed, with increased use of fertilizer but poor productivity growth (World Bank, 2006). The country is still vulnerable to recurrent food shortfalls and national food insecurity (Abate et al., 2011). For instance, between 1998 and 2012 the average number of Ethiopians in need of food assistance fluctuated between 3 million and 14 million (IRIN, 2012). The country ranks at 173th out of 187 nations in terms of Human Development Index (UNDP, 2013).

In Ethiopia, there is relatively large literature dealing with issues related to agricultural extension like adoption status of improved agricultural technologies (Feleke & Zegeye, 2006; Darcon & Christiaensen, 2007; Gebregziabher & Holden, 2011; Beshir, Emana, Kassa, & Haji, 2012 among others). Although these studies provided useful information on the rate of adoption and factors influencing adoption, rigorous impact evaluations of agricultural extension interventions are scanty (Anderson & Feder, 2007; Gebremedhin et al., 2009; Nega et al., 2010; World Bank, 2010).

Therefore, this study aims at evaluating the impact of agricultural extension program (AE) participation on smallholders' farm productivity using a plot-level data from three rural *kebeles* of Ethiopia. We started with a baseline model to estimate the impact of AE participation on farm productivity using Ordinary Least Square (OLS).Then we employed Heckman Treatment Effect Model (HTEM) and Propensity Score Matching (PSM) methods to address the problem of selection-bias due to self-selection of farmers into the program and endogenous program placement.

The rest of this paper is organized as follows. The next section describes a conceptual framework that illustrates the main tasks of the program in Ethiopia. The methodology section outlines the econometric procedures employed to estimate the impact of AE participation on farm productivity. Besides, it also outlines the sampling procedures of the study and type of data used for analysis. The results and discussion section provides and discusses the estimated impacts of participation in extension program on farm productivity. The last section summarizes the main findings, and draws some policy implications and outlook for further research.

2. Conceptual Framework

The conceptual framework illustrates how agricultural extension program that is used to enhance farmers' knowledge and skills, as well as promote and expand improved technologies affect farm productivity of Ethiopian smallholders. It is a general fact that, agricultural extension and advisory services play an important role in agricultural development and can contribute to improve the welfare of farmers and other people living in rural areas. In spite of this, there are many factors that condition the relationship between extension inputs and outcomes, and these factors act in complex ways.

According to Anderson and Feder (2003) productivity improvements are only possible when there is a gap between actual and potential productivity. They suggest two types of 'gaps' that contribute to the productivity differential, the technology gap and the management gap. Extension can contribute to the reduction of the productivity differential by increasing the speed of technology transfer and by increasing farmers' knowledge and assisting them in improving farm management practices (Feder, Murgai, & Quizon, 2004).

To make it understandable and consistent with the objective of this paper and the design of agricultural extension program in Ethiopia, the pathways showing how the extension program impact agricultural productivity are illustrated in Figure 1.

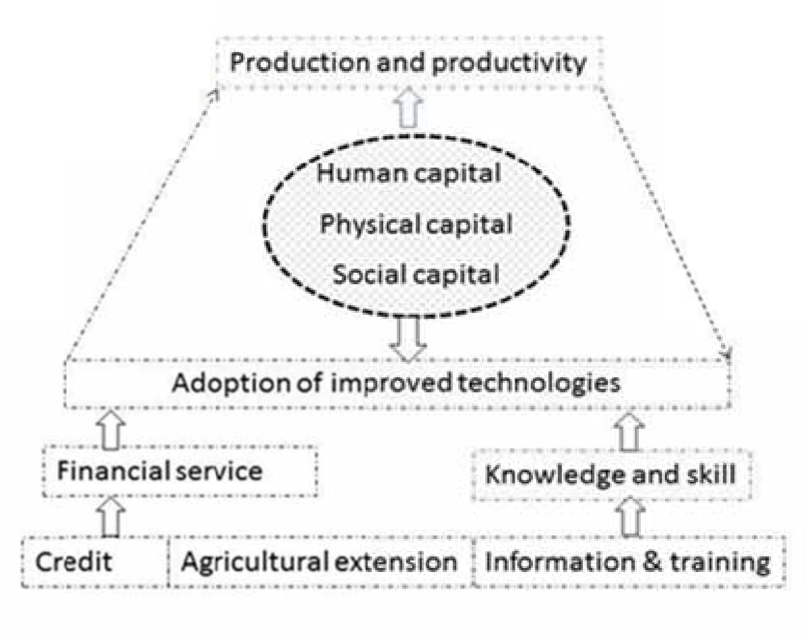

Figure 1. Impact pathway of agricultural extension on farm productivity

The mechanism to increase production and farm productivity through agricultural extension services are mainly tied with adoption of improved seeds, inorganic fertilizers, agro chemicals (herbicide and pesticide) and credit for the Ethiopian smallholder.

By facilitating credit services via cooperatives and microfinance institutions (e.g., credit in cash or in kind), the extension program enhances farmers' financial capacity which leads farmers to adopt improved technologies as well as practices that will ultimately meet their local farm production. Equally important, the extension service facilitates the adoption of improved technologies through awareness creation, acquiring knowledge and skill with dissemination of information and providing training that will ultimately help increase agricultural productivity. However, whether farmers actually adopt improved technologies and practices being promoted by the program is conditioned by several other household and farm level factors as well, such as human capital (sex, age, education level, and labour), physical capital (land size, livestock ownership), social capital (membership in farmers' organizations) and others (soil type, slope of the land, and farm management practices like intensity of ploughing frequency). The possible effect of each variable on land productivity has been hypothesized in Section 3.3.

3. Data and Methods

3.1 Description of the Study Area

This study was conducted in three selected *kebeles*[Note 1] (i.e., Enerata, Kebi and Wonka) in Gozamin *woreda* (district) of East Gojjam Zone in the Amhara Regional State, Ethiopia. It is one of the 17 administrative *woredas* in the Zone (Figure 2). It is found at about a distance of 265 km far from Bahir Dar city, the Regional capital, and at about 300 km from Addis Ababa city, the national capital. The *woreda* has a total area of 1,217.8 km^2 and an estimated population of 133, 856, out of which 98% are living in rural areas (CSA, 2008). There are 14 sector offices and 25 k*ebele*s in the w*oreda*.

Average annual rainfall of the *wereda* ranges from 1400 mm to 1800 mm and have an annual daily average temperature ranging between 11°C and 25°C. It covers three agro-ecological zones with 19% highland, 65% midland and 16% lowland (Gozamin *woreda* Administration Sector office documents, 2010). About 95% of total crop production is rainfall dependent (Benin, 2006). Generally, the district has a big potential for agricultural activities due to its agro-ecological diversification and dependable rainfall and optimum temperature.

The economy of the *woreda* is based on plough-based and labour intensive agriculture, which depends mainly on *meher* rain (main rainy season). Main crops grown in the *woreda* in order of abundance include *teff*[Note 2] wheat, maize, barely, check pea, soya bean, oats, niger seed (*Neug*) and lentil.

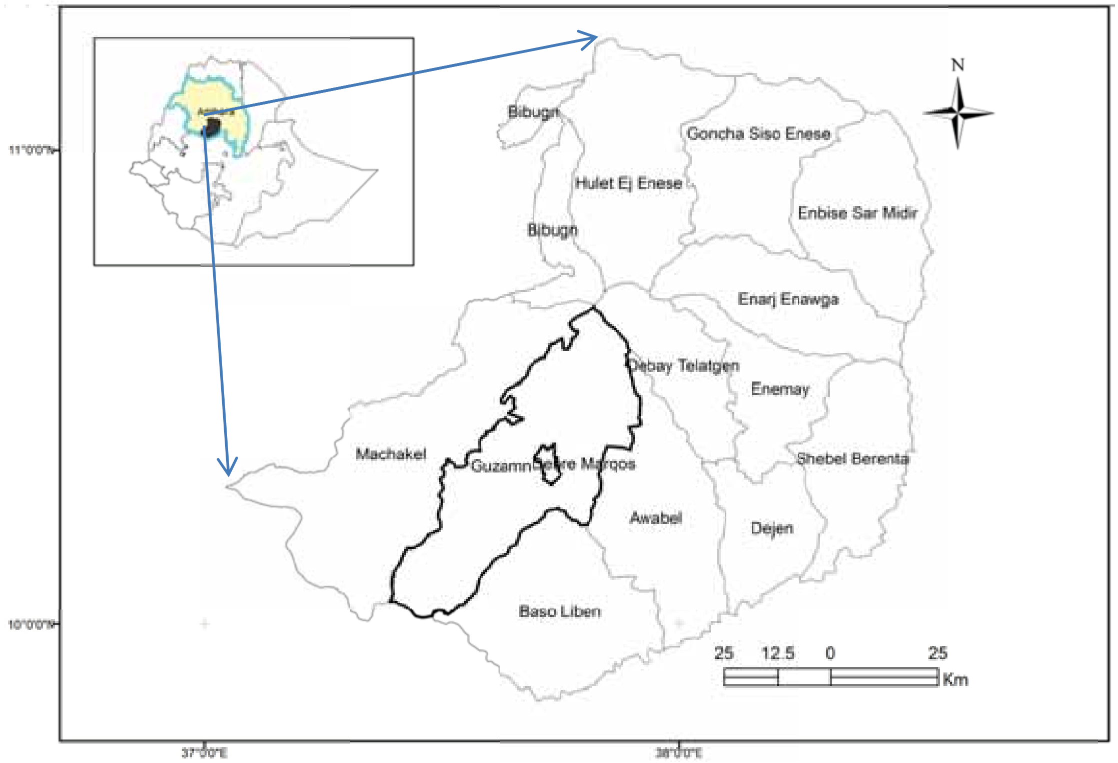

Figure 2. Location map of the study area

3.2 Data Sources

The data used in this study were obtained from a household survey conducted in three selected *kebeles* of Gozamin *woreda* in May and June 2012. The *woreda* was selected purposively for satisfying the following criteria; where crop production is widely practiced, where extension program have been implemented for relatively longer period of time, the availability of different agro-ecologies and its representativeness to the Ethiopian highlands. The Ethiopian highlands comprise nearly 45% of the total land area of 1.12 million square km, and support over 85% of the country's 82 million population that are overwhelmingly rural.

The three *kebele*s were randomly selected out of the total 25 *kebele*s found in the *woreda*. A multi-stage stratified random sampling technique was employed to select a total of 300 respondent farm households. First, farmers in each selected village were stratified into two groups as participant and non-participant of the extension program. The groups were identified from a list made available by the front-line extension workers, and then the information was confirmed by the farmers. Second, the two groups obtained from the first stage sampling were further stratified in to male and female headed households to ensure, as much as possible, representation of female-headed households in the sample. Data were collected both at household and plot level using structured and pre-tested questionnaire. Interviews and focus group discussions were used to compliment the data obtained through the field survey. The household data consists of demographic characteristics of household head, resource endowment, use of credit, membership in farmers' organization and involvement in *kebele* administration[Note 3].The plot level data consists of information on the intensity of input use (improved seed, inorganic fertilizer, compost and agro chemicals for pest and weed control), plot soil quality, plot slope, farm management practice (such as ploughing frequency) and amount of yield obtained during the 2011/12 main agricultural season for three selected crops (maize, wheat and *teff*) mainly targeted by the extension program.

3.3 Model Specification

This paper uses a combination of three methods (a benchmark Ordinary Least Square, Heckman's Treatment Effect and Propensity Score Matching) to assess the effect of participation in agricultural extension program on farm productivity.

3.3.1 The "benchmark" OLS Model

We start with a baseline model by estimating the impact of agricultural extension program (AE) participation on productivity using OLS.

The model is specified as follows:

$$Y_{ij} = \beta x_i + \psi x_j + \delta AE_i + \epsilon_{ij} \quad (1)$$

where, i and j denotes household and plot characteristics, the dependent variable Y_{ij} denotes the natural logarithmic transformation of gross value of crop produced per hectare (expressed in Ethiopian Birr/ ha)[Note 4], x_i is a vector of household level explanatory variables (sex, age, education level, labor, livestock ownership, membership in farmers' organization), x_j refers plot level explanatory variables (plot size, slope, soil fertility, amount of agrochemicals and inorganic fertilizer, compost, seed type, tenure type, draft power ,plot distance from home stead and ploughing frequency), β and δ are vector of parameters to be estimated, AE_i is a dummy variable indicating whether or not the ith household participate in the agricultural extension program and ϵ_{ij} is the error term. In this "benchmark" specification, the dummy has a constant coefficient, which gives the average treatment for the treated (ATT). The parameter δ measures the effect of AE program participation on farm productivity.

The effect of explanatory variables on the dependent variable has been hypothesized as follows. The variable age can be considered as an indicator of farming experience, on the other hand, those who are aged households may be reluctant to take up and apply improved technologies as a result the effect of age on crop productivity is ambiguous. Higher level of household education is likely to be associated with higher productivity because education enhances the ability of individuals to utilize technical information and such households would have better use of technologies and farming practice via access to information. On the other hand, there could be cases that educated households have high chance of engaging themselves in other non-farm related activities such as sideline business, involvement in *kebele* administration that would leave them with little time to spend on their farming activities. Regarding the sex of household head, most studies in developing countries report that female-headed households are the poorest and marginalized people due to their resource and other constraints such as access to credit, market information, assets, technical knowledge, cultural taboos and the likes. Hence we expect male-headed households would have better crop productivity than female headed households.

Physical capital or asset ownership which is usually used as a proxy to explain the wealth status of rural households can be explained by different variables. These are land and livestock which have been shown to overcome credit constraints in rural areas (Thirtle, Beyers, Ismael, & Piesse, 2003). The estimated coefficient is thus expected to be positive.

Social capital such as membership in farmers' organization might have indirect influence on productivity. This type of organization is mostly targeted by extension workers to disseminate information about improved technologies and farm practices. Therefore those farmers who are members of farmers' organizations might have greater chance to adopt technologies that lead to increase productivity.

OLS estimate of the coefficient for AE participation dummy is unbiased as far as participation is random. However, if the sample of the participants and non-participants is non-random, as it is often the case with non-experimental data like ours, OLS estimates of δ lead to a biased result. There are several approaches to deal with this problem (e.g. Heckman & Robb, 1985; Rosenbaum & Rubin, 1985; Angrist & Imbens, 1995; Wooldridge, 2010). The sample selection problem may arise from (1) self-selection where the households themselves decide whether or not to participate in extension program, due to differential resource endowments and/or (2) endogenous program placement where those who administer extension program (such as development agents) select households with specific characteristics (relatively poor or reasonably wealthy). As a result, extension participation may not be random that could give us a biased OLS result. To address the possible sample selection bias, we employed Heckman Treatment Effect and Propensity Score Matching techniques as discussed below.

3.3.2 Heckman Treatment Effect Model

One of the most widely used approaches to deal with selection bias is the Heckman treatment effect model. The Heckman correction, a two-step statistical approach, offers a means of correcting for non-randomly selected samples. The model can be specified in two steps:

Outcome equation:

$$Y_{ij} = \beta x_i + \psi x_j + \delta AE_i + \epsilon_{ij} \quad (2)$$

This is the same as the OLS equation in Equation (1)

Selection equation:

$$t_i^* = Z_i\gamma + \upsilon_i,\ t_i = 1 \text{ if } t_i^* > 0 \text{ and } t_i = 0 \text{ otherwise} \quad (3)$$

where t_i^* is the latent endogenous variable i.e., extension participation, υ is error term of the selection equation, Z_i is a set of exogenous variables predicting the selection of households into the extension program, ϵ_i and υ_i are bi-variate normal with mean zero and covariance matrix $\begin{bmatrix}\sigma_\epsilon & \rho \\ \rho & 1\end{bmatrix}$. Where ρ is the correlation between ϵ and υ, and σ_ϵ is the variance of ϵ. The inverse mills ratio, λ, is a product of this two i.e., $\hat{\lambda} = \hat{\sigma}_\epsilon \hat{\rho}$.[Note 5]

Selection equation: The probit model is estimated in which extension participation is regressed on a set of household characteristics Z_i. Variables included in the selection equation are: age of the household head (Age), total land holding of the household (LSize), owned livestock (TLU), family labor in adult equivalent (Adequv), distance from plot to extension center (Pdadist), number of oxen used (Oxenday) and a set of dummies indicating (i) whether the household head is educated (Educ) (ii) whether the household is member of *kebele* administration (Kebadm) and (iii) whether the household is member of farmers' organization (Frorg). Each of these variables is expected to only affect farm productivity through their impact on participation.

The extension program participation equation:

$$\Pr(\text{AEParticipation}_{i=1}) = \Phi[\gamma_o + \gamma_1\text{Educ} + \gamma_2\text{Kebadm} + \gamma_3\text{Frorg} + \gamma_4\text{Age} + \gamma_5\text{Pdadist} + \gamma_6\text{LSize} + \gamma_7\text{Adequv} + \gamma_8\text{TLU} + \gamma_9\text{Oxenday} + \upsilon_i] \quad (4)$$

The choice of the explanatory variables included in Z is guided by previous empirical literature on the decision of participation in development intervention programs.

Age can influence participation negatively or positively. Older farmers are often viewed as less flexible, and less willing to engage in a new or innovative activity due to fear of risk whereas young farmers may be more risk averse to implement new technologies on their farm. Hence, the influence of age on participation decision is ambiguous. Education might have positive contribution for participation in two ways. Either the farmers select the program due to their ability to understand the cost and benefit of participation in the program as well as easily understand how to implement new technologies (Doss & Morris, 2001) or extension program might target farmers who are educated due to their capacity of investing in improved technologies through participation in the non-farm sector (Barrett, Reardon, & Webb, 2001; Cunguara & Moder, 2011).

Wealth (land, livestock ownership, and family size in adult equivalent scale) might help farmers mitigate incomplete credit and insurance markets (Zerfu & Larsony, 2011; Ayalew & Deininger, 2012). Extension program may also target wealthier farmers due to their financial capacity to adopt improved technologies, and thus extension workers might want to deal with them to implement improved technologies promoted by the program.

In the study area, a hard-working and productive farmer is often described by the locals by how well he/she does the different farm activities starting from land preparation to post-harvest. The quality of doing these activities can better be estimated from the number of oxen days a farmer used at plot level, which was collected during our survey. Hence we used number-of-oxen days to characterize each farmer's commitment to farming and such kind of farmers might have high probability of participation in the extension program.

Membership in farmers' organizations can influence participation positively due to either extension workers might find it cheaper to target farmers group which helps them maximize the payoffs from efforts to build farmers capacity to demand advisory service (Benin et al., 2011; Cunguara & Moder, 2011) or membership in a social group provides opportunities to discuss and observe practices of other members at no cost or time intensity (Gebreegziabher, Mathijs, Maertens, Deckers, & Bauer, 2011).

Involvement in *kebele* administration could influence participation positively. One *kebele* consists of four to seven villages and these villages are often relevant units for government initiatives and program. A village in turn consists of *limat budin*[Note 6], or development team for the implementation of a range of government activities, including mobilizing household labor for community projects. They also have political functions, such as mobilizing support and votes for the ruling party. Extension workers often work closely with *limat budin* (Cohen

& Lemma, 2011; Birhanu, 2012). Hence, being in a position to involve in *kebele* administration with such kind of network system might increase the probability of participation in government sponsored extension program. We do not expect involvement in *kebele* administration to be correlated with farm productivity hence it might function as an identifying variable in the sample selection model.

The productivity equation is estimated in which farm productivity is regressed on a set of household and plot level characteristics. This is similar to those variables used in the OLS regression with additional regressor the Inverse Mill's Ratio (IMR) or Lambda (the residuals produced by the first-stage estimate of HTEM) included as a control variable in the productivity equation.

Outcome (farm productivity) equation:

$$\begin{aligned} Yield_{ij} = {} & \alpha + \delta AEParticipation_i + \beta_1 Sex_i + \beta_2 Age_i + \beta_3 Educ_i + \beta_4 TLU_i + \psi_1 PlotSize_{ij} + \psi_2 Slope_{ij} \\ & + \psi_3 Soilfertility_{ij} + \psi_4 Agrochemical_{ij} + \psi_5 Compost_{ij} + \psi_6 Fertlizer_{ij} + \psi_7 Seedtype_{ij} + \psi_8 Dist_{ij} \\ & + \psi_9 Tenuretype_{ij} + \psi_{10} Oxenday_{ij} + \psi_{11} Labour + \psi_{12} Ploughingfrequency_{ij} + \psi_{13} Cropdummy_{ij} \\ & + \psi_{14} Sitedummy_{ij} + IMR + \epsilon_{ij} \end{aligned} \quad (5)$$

where, i is household characteristics and j denotes plot characteristics.

However, a major limitation of the Heckman treatment model is that it imposes a linear form on the productivity equation and it extrapolates over the regions of no common support, where no similar participant and non-participant exist. But economic theory suggests that imposing such distributional and functional restriction may lead to biased result (Rosenbaum & Rubin, 1983; Dehejia & Wahba, 2002; Heckman & Navarro-Lozano, 2004). Therefore, we complement the analysis with semi-parametric matching approach (Rosenbaum & Rubin, 1985) to ensure the robustness of our previous model estimations.

3.3.3 Propensity Score Matching

Matching is a widely used non-experimental method of evaluation that can be used to estimate the average effect of a particular program (Smith & Todd, 2005; Caliendo & Kopeinig, 2008). This method compares the outcomes of program participants with those of matched non-participants, where matches are chosen on the basis of similarity in observed characteristics. Suppose there are two groups of farmers indexed by participation status P = 0/1, where 1 (0) indicates farms that did (not) participate in a program. Denote by y_i^1 the outcome (farm productivity) conditional on participation (P = 1) and by y_i^0 the outcome conditional on non-participation (P = 0).

The most common evaluation parameter of interest is the mean impact of treatment on the treated, $ATT = E(y_i^1 - y_i^0 | p_i = 1) = E(y_i^1 | p_i = 1) - E(y_i^0 | p_i = 1)$, which answers the question: 'How much did farms participating in the program benefit compared to what they would have experienced without participating in the program?' Data on $E(y_i^1 | p_i = 1)$ are available from the program participants. An evaluator's main problem is to find $E(y_i^0 | p_i = 1)$, since data on non-participants enables one to identify $E(y_i^0 | P = 0)$ only. So the difference between $E(y_i^1 | P = 1)$ and $E(y_i^0 | P = 1)$ cannot be observed for the same farm.

The solution advanced by Rubin (1977) is based on the assumption that given a set of observable covariates X, potential (non-treatment) outcomes are independent of the participation status (conditional independence assumption-CIA): $y_i^0 \perp S_i \mid X$. Hence, after adjusting for observable differences, the mean of the potential outcome is the same for P = 1 and P = 0, ($E(y_i^0 | P = 1, X) = E(y_i^0 | P = 0, X)$). This permits the use of matched non-participating farms to measure how the group of participating farms would have performed, if they had not participated.

Like the Heckman treatment effect model, propensity score matching has two-step. First, the propensity score (pscore) for each observation is calculated using logit model for AE participation (estimating a first-step equation similar to equation 3). The second step in the implementation of the PSM method is to choose a matching estimator. A good matching estimator does not eliminate too many of the original observations from the final analysis while it should at the same time yield statistically equal covariate means for treatment and control groups (Caliendo & Kopeinig, 2008). Hence, a kernel matching algorithm is used to pair each AE participant to similar non-participant using propensity score values in order to estimate the ATT. We also analyzed the data using alternative matching estimators to check the robustness of our results.

As explained above, the main assumption of PSM is selection on observables, also known as conditional independence or unconfoundedness assumption. Therefore, the specification of the propensity score is crucial because the logit model results depend on the unconfoundedness and overlap assumptions among others.

Unconfoundedness assumption implies that adjusting for differences in observed covariates removes bias in comparisons between the two similar groups that only differ by AE participation. In other words, beyond the observed covariates, there are no unobserved characteristics that are associated both with the potential outcome and the treatment (Imbens & Wooldridge, 2009). Although unconfoundedness is formally untestable, there are ways to assess its plausibility. To address the unconfoundedness assumption in this study, different measures are taken such as inclusion of many covariates in our propensity score specification to minimize omitted variables bias following the suggestion in Smith & Todd (2005), then matching is implemented on the region of common support (Heckman et al., 1997). In addition, we employed a placebo regression (Imbens & Wooldridge, 2009) as a robustness check of the impact estimates to unobserved selection bias. This approach was also used by Abebaw and Haile (2013) and Cunguara and Moder (2011) to test unobserved bias in the impact estimate.

The overlap assumption implies that the conditional distributions of the covariates of AE participants overlap completely with non-participants (Dehejia & Wahba, 2002; Imbens & Wooldridge, 2009). There are two formal methods of testing the overlap assumption. The first is to plot the distribution of the propensity scores of AE participants and non-participants and visually assess whether the overlap assumption holds or not. The second method is to compute normalized differences between the two groups (Imbens & Woolridge, 2009). The normalized difference is given by:

$$\Delta x = \frac{\bar{x}_1 - \bar{x}_0}{\sqrt{\sigma_1^2 + \sigma_0^2}} \tag{6}$$

where $\overline{x_\iota}$ is the mean, and σ_ι^2 is the sample variance.

4. Empirical Results

4.1. Descriptive Analysis of Bio-Physical and Socioeconomic Conditions

Tables 1 and 2 indicate a summary of descriptive statistics for household and plot level data respectively. Regarding demographic characteristics, the result revealed that, the average age for participant farmers were slightly less than non-participants. Literacy rate is significantly high for participant household heads (68%) than those who did not participate (24%). The average family size is 6.35 and 4.89 for participants and non-participants respectively. Available active family labor in adult equivalent for participants is 3.22 and 2.61 for non-participants. Average land holding size for participants is 1.53 and 1.05 hectare for non-participants. The average owned livestock size in TLU is 8.91 and 4.48 for participants and non- participants respectively.

Table 1. Descriptive statistics of household level data (n=300) used in the econometric analysis

	All sample		Participant		Non-participant		
Variables	Mean	Std.dev	Mean	Std.dev	Mean	Std.dev	P-value
Age of Household head (HH) (years)	45.70	10.07	45.45	10.02	46.09	10.14	0.303
Sex of HH (1 = male,0 = female)	0.82	0.38	1.00	0.00	0.54	0.49	0.000
Education of HH (1 = literate, 0 = illiterate)	0.50	0.50	0.68	0.47	0.24	0.43	0.000
Family size	5.77	1.92	6.35	1.81	4.89	1.74	0.000
Available family labor (adult equivalent)	2.98	1.06	3.22	1.03	2.61	1.01	0.000
Owned land size (hectare)	1.34	0.63	1.53	0.60	1.05	0.57	0.000
Owned livestock (Tropical Livestock Unit)	7.14	3.92	8.91	3.83	4.48	2.18	0.000
Use of credit previous year (1 = yes, 0 = no)	0.16	0.37	0.17	0.37	0.16	0.37	0.839
Number of training received for the last three years.	3.79	5.31	5.72	6.08	0.89	1.10	0.000
Membership in farmer's organizations (1 = yes, 0 = no)	0.81	0.39	0.96	0.21	0.58	0.49	0.000
Involvement in *kebele* administration work (1 = yes, 0 = no)	0.25	0.44	0.46	0.49	0.01	0.10	0.000

Table 2. Descriptive statistics of plot level data (n=1112) used in the econometric analysis

	All sample		Participant		Non-participant		
Variables	Mean	Std.dev	Mean	Std.dev	Mean	Std.dev	P-value
Value of crop produced per hectare (Birr/ha)*	12114	4721	13657	4834	9801	3431	0.000
Seed type (1 = improved, 0 = local)	0.43	0.49	0.53	0.50	0.27	0.44	0.000
Fertilizer used per hectare (kg/ha)	129.80	91.55	154.25	95.48	93.15	71.10	0.000
Compost used (1 = yes, 0 = no)	0.18	0.39	0.23	0.42	0.10	0.31	0.000
Chemical used per hectare (lit/ha)	0.29	0.55	0.36	0.63	0.18	0.39	0.000
Plot size (hectare)	0.28	0.15	0.29	0.15	0.25	0.14	0.000
Fertile soil (1 = fertile, 0 = otherwise)	0.24	0.43	0.24	0.43	0.23	0.42	0.725
Medium fertile (1 = medium fertile, 0 = otherwise)	0.58	0.49	0.58	0.49	0.58	0.49	0.874
Flat slop (1 = flat, 0 = otherwise)	0.32	0.47	0.32	0.47	0.33	0.47	0.779
Medium slop (1 = medium flat, 0 = otherwise)	0.54	0.50	0.52	0.50	0.58	0.49	0.057
Tenure type (1 = Owned, 0 = otherwise)	0.83	0.37	0.79	0.41	0.89	0.30	0.000
Plot distance from homestead (Walking minutes)	16.70	15.52	17.44	15.36	15.58	15.70	0.050
Amount of labor used per plot (person days/ha)	29.98	10.41	31.98	11.39	26.97	7.84	0.000
Amount of draft power used per plot (oxen days/ha)	20.03	10.21	22.45	10.83	16.39	7.96	0.000
Number of Ploughing frequency	4.92	1.41	5.11	1.41	4.63	1.38	0.000

*Average market prices were used to estimate aggregate crop production at the plot level, therefore production estimates are not affected by variation in local price.

Access to credit remains very low for majority of sample households. Only 17% of participants and 16% of non-participants had access to credit. About 96% of participants and 58% of non-participants were members of farmers' organization. Moreover, about 46% of participant farmers are involved in *kebele* administration whereas the non-participant's involvement in *kebele* administration is only 1%.

The average value of crop produced per hectare is 13657 Birr for participants and 9801 Birr for non- participants. The amount of inorganic fertilizer, chemical (pesticide and herbicide), and seed inputs used per hectare were computed from the actual amounts of those inputs used on each plots standardized to a per hectare level. Accordingly, the average intensity of fertilizer used per hectare by the sample households is 129 kg, 154 kg and 93 kg for all sample plots, plots of participant and non-participants respectively. This result is consistent with findings of Zerfu and Larsony (2011). The average intensity of chemical use rate by participants was 0.36 liters per hectare whereas non participants used 0.18 liters per hectare. Average plot size is 0.28, 0.29 and 0.25 for all farmers, participants and non-participants respectively. Generally the descriptive statistics result indicates that there is significant difference between participants and non-participants in terms of household characteristics, resource endowment, input use and productivity without controlling other factors. Therefore, our next question is what would happen if other factors are controlled? The different models used in this study could give the answer.

4.2 Ordinary Least Square Results (OLS)

The results presented in Table 3 show that participation in extension program leads to increased farm productivity by about 6%. However, to measure the benefit of participation in the program in terms of farm productivity, it is necessary to take in to account the fact that, individuals those who participated might have produced higher production even if they had not participated. That is, there may be unobserved factors (e.g., ability) that increases the likelihood of participation in the program that in turn increase productivity. When this is the case the impact of the program would be overestimated by simply regressing farm productivity on a binary variable that indicates participation in the extension program. To control this sample selection bias, we estimated Equations (4) and (5) together using treatment effect model and the result is presented in the following section.

4.3 Heckman Treatment Effect Model (HTEM) Results

4.3.1 Determinants of Extension Program Participation

The probit model for AE program participation shows that all variables except distance to extension center are significant determinants of participation in the current agricultural extension program. The model correctly predicted 70% of observed characteristics of participants and non- participants. The likelihood of participation in the extension program is affected significantly by age, education, livestock ownership, adult equivalent, use of oxen power, membership in farmers' organizations and involvement in *kebele* administration.

The negative and significant impact of household head age on the probability of joining the extension program indicates the lower likelihood of older farmer's participation in the program. This can be explained by the fact that older farmers are reluctant to accept new information and improved technologies. This result is consistent with studies reported by Genius et al. (2006). However, our result is contradicting with observations made by Tiwari et al. (2008); Mendola (2007). Hence, the impact of farmers' age on extension participation and/or technological adoption is ambiguous as expected. Education increases the probability of joining the extension program. This is consistent with the notion that farmers with better human capital like education are among the early adopters (Gebreegziabher et al., 2011; Giovanopoulou, Nastis, & Papanagiotou, 2011).

Table 3. Results of OLS and HTEM (Dependent variables: ln (value of crop produced/ha) and AE participation (1/0)

	OLS		HTEM		Probit	
Variables	Coef.	Std.err.	Coef.	Std.err.	Coef.	Std.err.
AE participation	0.0606**	0.0265	0.179***	0.0445		
Sex of HH	0.0685***	0.0256	0.0482*	0.0281		
Age of HH (ln)	-0.0847**	0.0427	-0.0753*	0.0418	-0.949***	0.289
Education of HH	-0.0132	0.0189	-0.0425**	0.0210	0.852***	0.116
Owned livestock (ln) (TLU)	0.0403**	0.0165	0.0061	0.0197	1.463***	0.145
Plot size (ln) (ha)	0.0917**	0.0366	0.0729**	0.0358		
Flat slop	0.0778**	0.0340	0.0758**	0.0306		
Medium slope	0.0308	0.0323	0.0317	0.0278		
Fertile soil	0.330***	0.0324	0.337***	0.0303		
Medium fertile	0.247***	0.0283	0.250***	0.0244		
Tenure type	0.0034	0.0253	-0.0074	0.0243		
Seed type	0.1830***	0.0231	0.1750***	0.0222		
Fertilizer (kg/ha)	0.0013***	0.0001	0.0013***	0.0001		
Compost	0.1260***	0.0314	0.1260***	0.0285		
Agro chemicals (lit/ha)	0.0307**	0.0153	0.0253	0.0161		
Plot distance from home stead(ln) (walkingminute)	0.0028	0.0091	0.0020	0.0088		
Draft power (ln) (oxen day/ha)	0.1550***	0.0459	0.150***	0.0410		
Labour (ln) (person day/ha)	0.1370***	0.0418	0.124***	0.0397		
Ploughing frequency	0.0216**	0.0097	0.0224**	0.0094		
Sitedummy_Enerata (cf:Kebi)	0.0267	0.0240	0.0255	0.0225		
Sitedummy_Wonka	0.1060***	0.0201	0.105***	0.0212		
Cropdummy_Wheat (cf:Maize)	0.0599	0.052	0.0523	0.0447		
Cropdummy_t*eff*	0.2080***	0.053	0.201***	0.0458		
Adult equivalent					0.178***	0.062
Owned land (ha)					0.321**	0.118
Kebele administration					1.572***	0.257
Membership in farmers' organizations					1.292***	0.172
Oxen days					0.428***	0.101
Plot distance from extension center (ln) (walking minute)					-0.115	0.077
Lambda			-0.0904***	0.0279		
Constant	7.477***	0.222	7.534***	0.212	-1.64	1.087
Observations	1112		1112		1112	
R-squared	0.567					
LR test of indep. eqns. (rho = 0): chi2(1) = 8.37 Prob > chi2 = 0.0038						

*** p<0.01, ** p<0.05, *p <0.1.

As hypothesized, all wealth indicator variables have significant effect on the probability of participation. One more tropical livestock unit increases the probability of participation in the extension program by about 14 %. Owned land and family size in adult equivalent scale also increases the likelihood of participation. One of the characteristics of Ethiopian agriculture is its labour intensive nature; hence, households who have large number of family size in adult equivalent scale have high likelihood of participation in the extension program.

As expected, use of intensive oxen power is positively significant with participation. This implies that farmers who are believed to be hard-working have a high chance of joining the extension program and other similar development interventions in a bid to improve their productivity. However, the measurement used to characterize a hardworking farmer is still a subject of refinement in future researches.

Membership in farmers' organization has positive significance for the probability of participation in the extension program as expected and consistent with past findings (Benin et al., 2011; Abebaw & Haile, 2013).

Involvement in *kebele* administration has the highest coefficient value among all the variables which affect the likelihood of participation. This implies that being affiliated with *kebele* administration, which is a nonfarm related activity, increases significantly the likelihood of farmers to join the extension program. This is due to lack of clear boundary between the extension program and the political administration which often share common human and material resources. For instance, as explained in our assumption, development agents often work closely with development team who are the major components of the *kebele* structure established by the government. The development team has also political functions, such as mobilizing support and votes for the ruling party (Cohen & Lemma, 2011; Birhanu, 2012). Hence, it is not surprising that being in a position to involve in *kebele* administration increases the probability of participation in government sponsored extension program. Previous studies show that involving in local administration facilitates access to credit and fertilizer because these supplies are channeled through local agencies (Ayalew & Deininger, 2012; Zerfu & Larsony, 2011). Furthermore other studies show that implementation modalities are given to local agencies, so that the system is potentially open to local influence (DSA, 2006).This fact is confirmed by World Bank (2010) report; politicians provide public services to clients in exchange for political advantage. This, in turn, leads to inequality in service provision, typically to the disadvantage of women and the poor.

4.3.2 Impact of Extension Program Participation on Farm Productivity

The result from HTEM in Table 3 shows that participation in AE increases farm productivity by about 20%. Unexpectedly the HTEM estimation for the effect of AE participation on productivity is higher compared to OLS estimation (6%), which was estimated without treating the endogenity of extension participation. The inverse mills ratio is negatively significant which indicates the presence of serious selection bias, due to the fact that program participants were selected by other nonagricultural related affiliations such as involvement in *kebele* administration (Table 3).

Other factors which have positive influence on farm productivity were sex of household head, age, plot size, soil quality, slope of the plot, use of improved seed, amount of inorganic fertilizer, application of compost, ploughing frequency, labour and oxen power. All significant variables have the expected signs. Male-headed households have 5% higher farm productivity than female headed households. The result is consistent with literatures which deals with the existence of gender variation in productivity (Pender & Gebremedhin, 2007) due to constraints related to labor, resource endowment, access to information and cultural taboo.

According to our result, as age increases farm productivity decreases. This could be attributed to the reason that getting older might pose disadvantages in agriculture because most of the work is physically demanding and also because older household heads might be too conservative to try new and more efficient techniques that could help to increase farm productivity. This result is consistent with the findings of GulUnal (2008) and Dong, Lu, and Featherstone (2010).

Despite the importance of education in increasing farm productivity (Alene & Manyong, 2007; Gebremedhin et al., 2009), surprisingly its effect was negatively significant. This could be partly attributed to the fact that educated farmers are involved in non-agricultural related activities (e.g. *kebele* administration in this study context), which would consume much of their farming time. However this is a tentative hypothesis to explain the unexpected result and needs further empirical study.

Plot size is positively significant with farm productivity. An increase in plot size by one hectare could increase yield by about 0.073%. This finding is consistent with earlier observations by Sharma et al. (1999), Lundvall and Battese (2000), and Alvarez and Arias (2004), who have all reported a positive relation between average land productivity and land size.

As expected, crop yield on fertile soil is higher due to the good quality advantage of such soils. Ploughing frequency has also significant positive effect for farm productivity. Similarly improved seed use increases productivity by 19%, indicating the relative importance of promoting improved seed to increase crop productivity in Ethiopia. Application of compost increases productivity by 13%. This reinforces the importance of soil fertility management in the Ethiopian agriculture. An increase in fertilizer use by about 50 kg/ha increases yield by about 7%.[Note 7]

4.4 Propensity Score Matching Results

As shown in Table 4, the propensity scores for each observation is calculated using logit model to predict the conditional probability of participation in AE program. The empirical model for AE participation correctly predicts 71.24% of the sample observations. The region of common support for the distribution of estimated propensity scores of participants and non-participants ranges between 0.014763 and 0.900497. Observations whose propensity score lies outside this range are discarded. The distributions of the propensity scores are plotted in Figure 3.

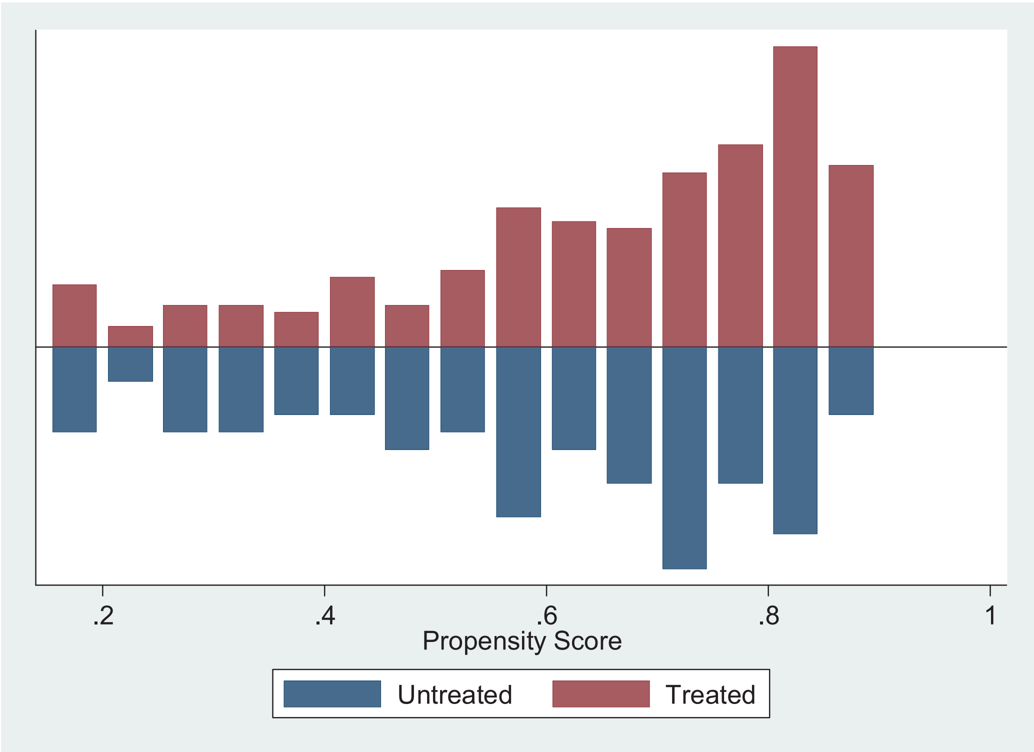

Figure 3. Propensity score distribution of matched samples

Most of the covariates in the logit model have the expected sign and comply with our previous result. The estimation results indicate that participation in AE program is strongly associated with the household's demographic characteristics and resource endowment as well as membership in farmers' organization and involvement in *kebele* administration. This result confirms again involvement in *kebele* administration, livestock ownership, and membership in farmers' organizations according to their importance order play a significant role on the likelihood of participation in extension program. From this, it can be generalized that the current agricultural extension program in Ethiopia is not targeting the majority poor. This finding is in line with the work by Lefort (2010) who reported that wealthier farmers are forcibly enrolled in the ruling party and appointed as model farmers who received privileged access to credit, state-controlled agricultural inputs, and technical knowledge spread by development agents.

Table 4. Estimation of the propensity score (Dependent variable: AE participation 1/0)

Variables	Coef.	Std.err.
Age of HH(ln)	-1.487***	0.541
Education of HH	1.479***	0.209
Owned livestock(ln)	2.582***	0.272
Owned land	0.419**	0.156
Adult equivalent	0.302***	0.108
Oxen days (ln)	0.745***	0.181
Plot distance from extension center(ln)	-0.154	0.145
Kebele administration	2.987***	0.526
Membership in farmers' organizations	2.348***	0.331
Sitedummy_*Enerata(cf:Kebi)*	-0.009	0.259
Sitedummy_*Wonka*	-0.068	0.243
Constant	-3.820*	2.048
Observations	1112	
Pseudo R^2	0.5354	
Model prediction rate: 71.24%		

*** p<0.01, ** p<0.05, *p<0.1.

4.4.1 Average Treatment Effect on the Treated

The PSM method is employed in estimating the impact of participation in agricultural extension on farm productivity. The impacts are estimated using alternative estimators to ensure robustness. As indicated in Table 5, all the matching estimators show that participation in agricultural extension program has a positive and statistically significant effect on farm productivity. To ensure the reliability of the estimated results, assessment on the overlap and unconfoundedness assumptions are made.

Table 5. Estimating the ATT using different matching methods

Matching estimators	Coefficient	t-statistics
Kernel matching	0.203***	4.738
Stratification matching	0.190***	3.289
Radius matching	0.175***	2.763
Nearest Neighbor matching	0.327***	3.288

Significance levels are based on bootstrapped standard errors with 50 replications.

*** p<0.01.

4.4.2 Assessment on the Overlap and Unconfoundedness Assumptions

To evaluate the overlap assumptions we checked whether the balancing requirements of PSM are satisfied in our data. The balancing test in Table 6 indicates that the covariates of the two matched groups are well balanced in contrast to the unmatched samples presented in Table 1. All results of normalized differences between the two matched groups are small, suggesting that the overlap assumption is reasonable. Imbens and Wooldridge (2009) consider a normalized difference greater than 0.25 (in absolute value) to be substantial to detect any lack of overlap. In addition as shown in Figure 3 the two groups have substantial overlap in their propensity score distribution.

The placebo regression (Table A1) was employed using age of spouse of the household head as a dependent variable including AE participation and similar variables used in the estimation of the propensity scores. The dependent variable is known a priori not to be caused by AE participation. The result shows that AE participation does not have influence on the dependent variable, suggesting that there are no significant omitted variables that affect the impact estimates obtained by PSM method. Therefore, the unconfoundedness assumption can be maintained and the causal interpretation of the results is plausible.

Table 6. Balancing test of matched samples

Variable	AE participants		Non-participants		Normalized
	Mean	Standard deviation	Mean	Standard deviation	difference (Δx)
Age of HH (ln)	3.823	0.258	3.823	0.206	0.00
Education of HH	0.451	0.498	0.377	0.487	0.07
Owned livestock (ln)	1.955	0.361	1.84	0.336	0.13
Owned land size	1.411	0.539	1.369	0.647	0.03
Adult equivalent	3.148	1.037	3.043	1.004	0.07
Oxen days (ln)	2.897	0.520	2.825	0.521	0.06
Plot distance from extension center (ln)	3.426	0.654	3.354	0.721	0.05
Kebele administration	0.040	0.199	0.018	0.135	0.03
Membership in farmers' organizations	0.977	0.148	0.918	0.275	0.07
Sitedummy_*Enerata(cf:Kebi)*	0.294	0.456	0.295	0.458	0.00
Sitedummy_*Wonka*	0.406	0.492	0.336	0.475	0.13

Generally, all the estimated results obtained from the different models confirm that AE participation in the study area have increased farm productivity. However, the overall level of farm productivity observed in this study for the three case study crops (*teff*, wheat and maize) is still low compared to the target yield set by the regional extension program based on farmers' field conditions and research stations (Table 7). For instance average *teff* yield observed form extension participants (16 quintal/ha) is less by half from the extension targets (20-32 quintal/ha). Similarly, the yield levels attained by participant farmers for wheat and maize were less by 1/3 from the set target for the corresponding crops (43-58 quintal/ha and 70-107 quintal/ha). Several reasons could explain these discrepancies. Our field investigation and review of past researches (Abate, 2007; Kasa, 2008) show that the extension implementation in Ethiopia is constrained by a number of factors such as supply-push rather than demand-pull approach, poorly organized technology multiplication system, absence of institutional pluralism, low technology adoption rate, shortage of basic training for extension staff and others. For example in our study average application of fertilizer, improved seed and compost use rates were 129 kg/ha, 42% and 18%, respectively, which are much lower compared to the recommended rates. Besides, credit users in the study area were only 16%, influenced by the nature of credit arrangements that reduces the attractiveness of input uptake. To be eligible, a farmer must have repaid all previous loans (Dercon, 2000). Inconvenient payback time and lack of interest due to the tendency of farmers to avoid risk in instances of crop failure are other factors for farmers' low use of credit (Carlesson, Kohlin, Mekonnen, & Yusuf, 2005). Farmers who participated in our group discussion explained lack of quality improved seed, high price of fertilizers, limited technology choices and inconvenient loan system are the major constraints to adopt improved technologies promoted by the extension program. Furthermore our focus group discussion and field survey revealed that no single farmer has been visited by researchers implying the missing link between research and extension.

Table 7. Comparative average yields (quintal/hectare) of the three main crops grown in the study area

Type of crops	Participants' yield	Targeted yield on farmers plot	Yield obtained from research stations
Teff	16	20	32
Wheat	21	43	58
Maize	25	70	107

Average yield obtained by participants is calculated from sample plots taken by our study. Average targeted yield on farmers plot and yield obtained by research station is taken from a guideline compiled by Agriculture and Rural Development Office of the Amhara National Regional state (2011).

5. Conclusion

This study evaluates the effect of agricultural extension program participation on farm productivity using cross-sectional data collected in three *kebeles* from the Ethiopian highlands. Even though the overall impact of extension program participation cannot be known for certain because of the lack of reasonably accurate baseline data for comparison, this study employs a bench mark OLS, treatment effect model and propensity score matching methods to mitigate some of the challenges in the estimation of effect of agricultural extension participation on farm productivity.

Our model estimations indicate the positive effect of extension participation on farm productivity. However, in spite of its positive effect, our finding clearly shows the existence of selection bias which tends to target relatively wealthier farm households and those affiliated to *kebele* administration, which is not directly related to farm productivity. Furthermore, the program has been constrained by insufficient and/or poor quality farm inputs, such as selected seeds, and services like credit and training. As a result, the observed overall farm productivity is less by about half than the target set by the extension program.

Therefore, in order to improve the benefits to be gained through agricultural extension program participation, the following constraints need serious consideration. First, the extension program should avoid entry barriers and this requires maintaining a clear boundary between the program and the local politics which is missing at the moment. Second, improved access to diversified and quality agricultural inputs still remain critically important. Third, the local government should create the necessary asset portfolio among the poor due to the fact that resource poor farmers in Ethiopia lack the necessary means to implement extension advices.

We acknowledge, however, that our results cannot be generalized at the national level since the sample was not representative of the entire country. Hence to get more representative figure about the impact of the program at national level conducting similar studies further dealing with a wider sample size coverage and time series data that considers other aspects of the national extension program is important.

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Appendix A

Table A1: Placebo regression result

Dependent variable: age of head's spouse	Coefficient	Standard error	P-value
Agricultural extension participation	0.554	0.653	0.397
Age of HH(ln)	36.37	1.443	0.001
Education of HH	-0.17	0.517	0.742
Owned livestock(ln)	0.394	0.805	0.625
Owned land	0.311	0.502	0.537
Adult equivalent	0.122	0.258	0.637
Oxen days (ln)	0.012	0.474	0.979
Plot distance from extension center(ln)	-0.473	0.416	0.256
Kebele administration	-0.161	1.832	0.930
Membership in farmers' organizations	-1.418	1.752	0.419
Sitedummy_Enerata(cf:Kebi)	1.789	0.676	0.008
Sitedummy_Wonka	1.602	0.606	0.009
Constant	-99.52	5.21	0.003
Number of observations	366		
F(12, 353)	142.87		
Prob> F	0.000		
R squared	0.81		

Notes:

Note 1. *Kebele* (often translated as peasant association) is the lowest administrative unit in Ethiopia and usually.

Note 2. *Teff* is a small grain crop widely consumed in Ethiopia and is the main ingredient in injera (pancake-like food).

Note 3. *Kebele* administration, consists of an elected *kebele* council (in principle 100 members), a *kebele* cabinet (usually comprises a manager, chairperson, agricultural extension workers, school director, representatives from women and youth associations), a social court and security persons posted in the *kebele*. The *kebele* council and executive committee's main responsibilities are preparing annual *kebele* development plan, ensuring the collection of land and agricultural income tax, organizing local labour and in kind contributions to development activities, resolving conflicts with in the community (Yilmaz and Venugopal, 2008).

Note 4. Birr is Ethiopian currency and during the survey period 1US$ = 17 Birr.

Note 5. The treatment effect assumes non-zero correlation between ϵ and υ and hence violation of this assumption can lead to biased estimation.

Note 6. Previously called mengstawi budin, or government team.

Note 7. Semi-elasticities are estimated using the following formula: $\left[exp^{(\beta_{j\Delta}x_j)} - 1\right] * 100.$

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Yield Responses of Maize to Organic and Mineral Fertilizers at Different Inclinations in Tropical Smallholder Farming Systems

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Abstract

A field study was conducted on the potential of Gliricidia (*Gliricidia sepium* (Jacq.) Kunth ex Walp.) to enhance productivity of degraded soils. Maize was cropped in a hilly region of Sri Lanka with and without the recommended mineral fertilization, in two major seasons, October-January in 2007/8 (Year 1) and in 2008/9 (Year 2) on 92 farms at two inclinations: Flat (0-10%) and Moderate (10-30%). On half the farms, green manure (Gliricidia leaves) was added (3 tonnes per hectare per season). NPK boosted production to a very respectable mean grain yield of 4.2 t/ha on Flat farms. At ZERO, the yield was lower by 60%, irrespective of the inclination. Gliricidia failed to replace the required nitrogen, even with an adequate supply of phosphorous and potassium (PK). In contrast, together with NPK, Gliricidia increased yields by 15-20% compared to NPK alone, while the gain was 35% at ZERO. Fields in the Moderate category were more responsive to green manure and mineral fertilizers. The high response to mineral fertilizers indicated that the degradation of the soils resulted to a greater extent in chemical rather than in physical deficits. But intensive cropping reduced the soil organic matter within two years, to some extent slowed down by Gliricidia green manure. Therefore an intense cropping for the sake of food security must be accompanied by soil conserving cropping systems.

Keywords: degraded soils, tree legumes, green manure, inclinations, mineral fertilizers

1. Introduction

Due to continuous cultivation and soil erosion soil fertility often declines in tropical regions, jeopardizing food production on smallholder farms (Kwesiga et al., 2003; Akinnifesi et al., 2006). Agroforestry is a valuable alternative in such systems for regenerating soil fertility, and thus enhancing food security and household income, especially where mineral fertilizers are too expensive for smallholder farmers (Mafongoya et al., 2006; Kimaro et al., 2008). Trees in agroforestry offer diverse advantages like biological nitrogen fixation, nutrient recycling from deeper soil layers and minimization of leaching and soil erosion (Nyadzi et al., 2003). A number of studies revealed the benefits of Gliricidia for soil fertility (Ikerra et al., 1999; Chiwara et al., 2003; Makumba et al., 2005; Akinnifesi et al., 2009). Its green manure can contribute N (and other recycled nutrients) by 20-65 kg/ha in smallholder farming systems (Zingore et al., 2003; Baggie et al., 2004; De Costa et al., 2005).

Repeated green manure applications can provide more long-term (Sileshi & Mafongoya, 2006; Kimaro et al., 2007; Makumba et al., 2007) or more short-term (Sangakkara et al., 2004; Reddy et al., 2008; Silva et al., 2008) benefits. However, there are not always positive effects (Pandey & Rai, 2007; Marin et al., 2007). A meta-analysis by Sileshi et al. (2008) showed a lack of quantitative synthesis in terms of the nature and magnitude of green manuring and also the linkages among location, soil type and climate. In a recent study, 30 twinned focal points, i.e. a homegarden with long-term intensive application of green manure and a field, were compared with regard to the content of soil organic matter and maize yield (Egodawatta et al., 2012). Both parameters were much higher in homegardens than in fields, revealing that the annual application of Gliricidia green manure together with mineral fertilizer increased yields mostly in homegardens.

An agroforestry system based on Gliricidia was introduced into a mountainous rural region of Sri Lanka in 2001/2002 with the objective of replenishing the degraded landscape and simultaneously providing direct and indirect economic benefits. Farmers were encouraged to use green manure as part of their agricultural practices and to integrate nutrient management by synchronizing the application of mineral fertilizers and organic constituents.

The hypothesis was that Gliricidia green manure can increase the production potential of the model crop maize, irrespective of the terrain. The specific objectives were: 1) to assess the impact of Gliricidia manure on soil productivity, taking into account inclination and 2) to compare the impact of Gliricidia green manure and a recommended supply of mineral NPK fertilizer on the growth and yield of maize.

2. Method

2.1 Study Area and Experimental Sites

This on-farm study was conducted from 2007 to 2009 (Year 1 and Year 2) on 92 fields in the Meegahakiula region at 7°07.485'N, 81°02.740'E and at elevations between 270-400 MSL covering a total area of 25 km^2. The pattern of rainfall in the region is bi-modal, with an annual 1300 mm; about 60% is available for maize cultivation in the major season from October to January. The actual annual rainfall was 1325 mm (Year 1) and 919 mm (Year 2).

The absolute inclination of the 92 farms was measured (Bandara et al., 2011) and categorized as "Flat" (0-10%) and "Moderate" (10-30%). Initially, 27 farms at steeper inclinations were measured as well, but the cultivation practises often differed from those in the Flat and Moderate categories. Anyhow, sustainable arable cropping cannot be recommended for those steep areas (Egodawatta et al., 2012). Based on the use of Gliricidia, a tree legume crop, farms were further categorized as "G YES" and "G NO", depending on whether or not Gliricidia green manure was applied during cropping. Before the experiment started and after two years of cropping, with the same amount of Gliricidia manure being applied twice a year to the maize crop and to the subsequent crop of mungbean (*Vigna radiata*) in the minor rainy season (data not recorded here), a soil analysis was carried out for an easy determination of chemical and physical properties (*i.e.* pH, total nitrogen, available phosphorus, extractable potassium, organic matter, texture and bulk density) prior to crop establishment.

2.2 Experimental Set Up and Plot Management

With the onset of monsoonal rains, the existing vegetation was slashed to the ground, and all the debris was removed. The selected lands were ploughed with a conventional plough at an effective depth of 20 cm. Maize seeds of the local variety Ruwan (OPV, open pollinated variety) were planted into mounds, spaced at 30 cm x 45 cm, one seed per point to obtain a planting density of 7.4 plants/m^2. Plots were kept weed-free until four weeks after planting. All the cultivation practices followed local recommendations (Department of Agriculture, Sri Lanka, 1995). The three mineral fertilizer treatments were established in plots of 10 m x 10 m, randomly established within in each field: 1) total recommended fertilizer dosage (NPK), 69 kg N (Urea), P 20 kg (Triple Super Phosphate) and K 50 kg (Muriate of Potash) per hectare, 2) recommended fertilizer dosage without N (PK), and 3) no fertilizer (ZERO). On G YES farms, 3 t/ha (dry weight basis), half the Gliricidia leaves were incorporated during preparation of the land for planting and half as surface mulch four weeks after planting, amounting to 24 kg N, 3 kg P and 18 kg K per hectare and season.

2.3 Observed Parameters

A number of parameters (plant height, leaf number and SPAD values) were assessed during the vegetative period of maize at two-week intervals. The final analysis of the data proved that none of these parameters reflected the impact of factorial combinations better than the maize yield itself, and therefore the data are not shown. At full maturity, 10 m^2 were harvested to determine the grain yield. After harvesting the ears, the plants (without roots) from 1 m^2 were taken from each plot and separated into stover and cobs. The shoots were oven-dried at 60°C for 48 hours to determine the dry weight and leaf nitrogen. Total biomass, grain yield and harvest index were determined based on the collected data. The yield components (cobs per plant, rows per cob, number of kernels per row, and 100 kernel weight) were determined using the harvested sub samples. Since grain yield best explained the impact of the treatments, the data of all related parameters are not shown.

2.4 Data Analysis

Prior to the detailed analysis, the maize yield data was tested for normality, autocorrelation and homoscedasticity. A general linear Model (GLM) and ANOVA were carried out to assess the effects of inclination, year and Gliricidia on the model crop maize. By means of ANOVA, significant means were separated by the least significant difference (LSD) test. Pearson correlation coefficients were used to identify the relationships between soil and yield parameters. All analyses were conducted at the 5% level of significance. Appropriate statistical tools from SAS version 9.2 (SAS Institute, 2008) were used to perform the analysis.

3. Results

At the upper 30cm soil level available phosphorous (P) ranged between 24-26 mg/kg, and extractable potassium (K) between 15-18 mg/kg; detailed data is exemplarily shown in Table 1 for soil organic matter (SOM) total nitrogen (TN). Their values ranged between 12.8-16.5 g/kg, between 10.6-12.9 mg/kg, respectively. All ranges were generally narrow and differed little when comparing Flat and Moderate inclinations. SOM values significantly declined after two years of cultivation, irrespective of inclination and of Gliricidia green manure (G YES) addition. However, in the latter case the decline was slowed down. Opposite to SOM, TN values had increased over the two years of cropping (Table 1); a partly significant positive impact of green manure addition existed but it was less pronounced than for SOM values.

Table 1. Soil chemical and physical properties of top soil (0-30cm) in the inclination classes with use of Gliricidia as green manure (G YES and G NO) in 2007 and 2009

Soil Property	G use	Flat (*n=52*)		Moderate (*n*=40)	
		2007	2009	2007	2009
SOM (g/ kg)	G YES	16.5^{ax}	15.0^{ay}	16.4^{ax}	15.0^{ay}
		$0.8^{\dagger}$	0.6	1.1	0.9
	G NO	16.6^{ax}	13.3^{ay}	15.6^{ax}	12.8^{ay}
		0.8	0.6	1.0	0.7
Total Nitrogen (mg/ kg)	G YES	12.2^{ax}	12.9^{ax}	10.6^{bx}	12.7^{ay}
		0.4	0.4	0.1	0.5
	G NO	10.7^{ax}	11.5^{ay}	10.6^{ax}	12.3^{by}
		0.4	0.4	0.3	0.3

n = number of replicates in each inclination;

† standard deviation with corresponding mean;

Means followed by the same letters in a row are not significantly different at $p < 0.05$;

a, b: denote the difference between inclination categories, x,y : denote the difference between two years.

The statistical model for grain yield represented 57% of the total associated variability. Grain yield was only marginally ($P = 0.051$) influenced by year and, thus, it was not considered in the model. The differences between the years were mainly due to the small amount of rainfall in Year 2. The relative differences in yield due to fertilizer treatments were within a similar range at each inclination in both years. The two-way interactions (inclination and season, inclination and fertilizer combination, season and fertilizer combinations) or the three-way interaction (inclination and season and fertilizer combination) were not significant ($P < 0.05$). NPK, PK and ZERO fertilizer treatments significantly ($P < 0.001$) influenced grain yield at both inclinations (Table 2). At ZERO yields ranged around a subsistence level of about 0.9 to 1.7 t/ha and were mostly lower at Moderate than at Flat. PK raised the yield generally by more than 1 t/ha. Adding N, i.e. NPK, increased yields by a further 33 to 36%, and the combination NPK, Flat and G YES resulted in a respectable yield of 4.4 t/ha for an OPV. In general, yields were somewhat higher at Flat than at Moderate. G YES improved yields compared to G NO by about 15 to 20% at both NPK and PK and by about 35% at ZERO. Correlations between maize grain yields and soil properties were calculated on the basis of the single combinations of inclination category, use of Gliricidia, and fertilizer application. The respective values were mostly low but positive for SOM, TN and K. Some significant correlations existed in the combination Flat and G NO, with positive R-values of about 0.5 for P and negative ones of about 0.6 for bulk density for all three mineral fertilizer combinations.

Table 2. Mean grain yield (t/ha) of maize at two ranges of inclination with the use and non-use, or not, of Gliricidia (G YES or G NO) as green manure

Inclination category			Flat (0-10%) (*n=52*)			Moderate (10-30%) (*n=40*)		
Use of Gliricidia			G YES (*n=30*)	G NO (*n=22*)	% diff	G YES (*n=19*)	G NO (*n=21*)	% diff
Fertilizer combination	NPK		4.37* 1.06†	3.67 1.53	19	3.57* 1.57	3.10 1.38	15
	PK		2.84* 1.03	2.36 1.17	20	2.40* 1.18	2.07 1.17	16
	ZERO		1.65* 0.67	1.20 0.84	37	1.25* 0.67	0.93 0.71	34
	% diff	NPK-PK	35	36		33	33	
		NPK-ZERO	62	67		65	70	

n= number of replicates in each range of inclination;

† standard deviation associated with corresponding mean;

* significant difference at $P < 0.05$ (G YES and G NO) at the corresponding inclination and fertilizer combinations following the mean separation by least significant difference (LSD).

4. Discussion

The soil fertility status of the farms was heterogeneous, which led to a highly variable crop response and final grain yields of maize, even at the same inclination range, partly due to the diversity of management practices by individual farmers. This strongly highlights the advantage of multi-locational on-farm studies to avoid generalisations that are not justified in regions of varied topography. Generally, intensive cropping with a long and a short season crop per year reduced the SOM values within two years, a process that could be just slowed down by Gliricidia green manure. This indicated that an intense cropping must be accompanied by soil conserving cropping systems in the long term. In addition to soil parameters, different social status and income may have caused deviations in the correlations between soil quality and yield and requires further study, although the heterogeneity of crop yields can usually be linked directly to the fertility of the field (Mairura et al., 2007). Despite relatively small differences in the chemical soil parameters, yields were considerably higher at Flat than at Moderate. In Thailand and Australia the relative yield reduction at greater inclinations was lower (Sipaseuth et al., 2007; Cotching et al., 2002), but the annual rainfall was higher (2,000 mm) in Thailand, whereas the soil was more fertile in Australia. Therefore, attention must be paid to other factors that influence growth, not only to inclination.

Flat farms showed higher mean yields, especially at recommended levels of NPK. However, seasonal application of Gliricidia green manure still caused a significant increase in grain yields. It had been assumed that much of the impact of green manure on yield was due to nitrogen release. However, this assumption was largely negated by an identical response to green manure at PK, where available nitrogen is lower. Neither P and K nor N were sufficiently increased with the application of Gliricidia during the vegetative growth of these crops, even though the addition of Gliricidia should have resulted in the release of more than one third of the mineral N. Only at Zero was the relative response to G Yes higher, but since the absolute yield increase in comparison to NPK and PK was lower, the direct utilization of the nutrient contents in the green manure was clearly insufficient. In a parallel study, the maize yield of fields at selected farms was compared to the yields achieved in home gardens, which had received large amounts of organic manure, including Gliricidia, over long periods of time (Egodawatta et al., 2012). The higher yield potential of homegardens was probably due, not only to mineral fertilizers and the seasonal application of Gliricidia manure, but also to a generally greater fertility of the soil. These findings indicate that the long-term improvement of soil fertility was more important than short-term impacts of nutrients in our study (cf. literature review in the Introduction). Despite comparatively high P availability in the studied soils, the variability on the individual farms was still important for the exploitation of the yield potential, as indicated by the positive correlations for different combinations of fertilization and inclinations. Across farms for each range of inclination and mineral nutrient fertilization, the high impact of NPK and PK indicates that the chemical soil fertility was not exploited at ZERO, so that there is considerable room for increasing yield when and if it becomes economically feasible in the future. Confirming a report by FAO (2006), nitrogen was a key factor in achieving higher yields; this was corroborated too for more fertile home gardens at much greater inclinations (Wijesinghe et al., 2009; Egodawatta et al., 2012). However, even a high rate of N was not enough to elevate yields on farms at Moderate compared to yield at Flat, which was especially pronounced in the rather dry second season. Without addition of green manure, physical restrictions (Bulk density) and chemical restriction (P) were indicated in the former case by significant correlations to grain yield.

5. Conclusions

The production potential of maize was limited by a low content of soil nitrogen, as indicated by the strong response to the application of mineral N. The combined application of both fertilizer and green manure usually had a mutual effect on crop yields. Though the direct contribution of N by green manure was low, there seemed to be an indirect influence on chemical and physical soil parameters like SOM, bulk density and P, an important contribution to yield consistency in regions where "self-supply and food security " is of great importance.

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Inhibition of *Listeria* and *Staphylococcus aureus* by Bovicin HC5 and Nisin Combination in Milk

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Abstract

The aim of this work was to evaluate the effect of the bacteriocins bovicin HC5 and nisin against *Listeria* and *Staphylococcus aureus* in synthetic media and in milk. Growth of *Listeria monocytogenes*, *Listeria innocua* and *S. aureus* was carried out at 37ºC in tryptic soy broth (TSB) and in ultra-high temperature whole milk containing bovicin HC5 and nisin added either individually or in combination. Concentrations above 100 AU ml^{-1} of bovicin HC5 or 50 AU ml^{-1} of nisin inhibited the growth of *Listeria* species in TSB. Bacteriocins at concentrations of at least 50 AU ml^{-1} clearly increased the lag phase, but did not prevent the growth of *S. aureus*. The combination of both bovicin HC5 and nisin in TSB inhibited the growth of *Listeria* and *S. aureus* Embrapa 4018 at lower concentrations than the bacteriocins added separately. Bactericidal effect against *L. monocytogenes* and *S. aureus* cells was observed when both bacteriocins were added together in milk in concentrations larger than 400 AU ml^{-1} of each one. The present results demonstrate that bovicin HC5 and nisin were effective against *Listeria* and *S. aureus* assessed in milk, especially when used in combination.

Keywords: food pathogen, bovicin HC5, nisin, milk

1. Introduction

Outbreaks of listeriosis resulting from consumption of dairy products contaminated with *L. monocytogenes* have prompted concern about the behavior of this microorganism during processing and subsequent storage of various dairy products (Silva, Almeida, Alves, & Almeida, 2003). *L. monocytogenes*, a ubiquitous foodborne pathogen, can be potentially introduced in raw milk in a dairy industry environment. Growth of *Listeria* in dairy products is often favored by its psychrotrophic nature and tolerance to high salt concentration and relatively low pH values (Farber & Peterkin, 1991). *L. monocytogenes* causes disease in high-risk groups, including pregnant women, neonates, and immunocompromised adults, and has a high mortality rate (Arques, Rodriguez, Nunez, & Medina, 2008).

Food from animal origins, such as milk, is naturally susceptible to contamination by *Staphylococcus aureus*, an important pathogen able to grow in a wide range of temperatures, pH and sodium chloride concentration up 15%, and then able to produce enterotoxins. These toxins are thermostable and maintain their stability even after thermal treatments (Dinges, Orwin, & Schlievert, 2000; Le Loir, Baron, & Gautier, 2003).

Although *Listeria* and *S. aureus* are inactivated under normal conditions of pasteurization, problems can arise from post-pasteurization contamination, representing a risk to consumers, making necessary an effective control during the steps of food production.

Bacteriocins from lactic acid bacteria are widely studied and have been suggested as a potential biological alternative to improve food safety (Cleveland, Montville, Nes, & Chikindas, 2001). Nisin is a well known broad spectrum bacteriocin that can inhibit gram-positive bacteria and prevent the outgrowth of spores of Bacilli and Clostridia associated with food (Arques et al., 2004; de Arauz, Jozala, Mazzola, & Penna, 2009). Although nisin has been widely used in food industries to increase the shelf life of food products, previous studies indicated that many sensitive gram-positive bacteria have developed resistance to nisin (Arques et al., 2008; Zapico, Medina, Gaya, & Nunez, 1998).

Bovicin HC5, a bacteriocin produced by *Streptococcus bovis* HC5, has a broad spectrum of activity (Mantovani, Hu, Worobo, & Russell, 2002). Previous works demonstrated the ability of bovicin HC5 to inhibit *L. monocytogenes* (Mantovani & Russell, 2003), and prevent the growth and spore germination of strains of *Bacillus cereus* and *Bacillus thuringiensis* (de Carvalho, Costa, Mantovani, & Vanetti, 2007), *Clostridium tyrobutyricum* (de Carvalho, Mantovani, & Vanetti, 2007), and *Alicyclobacillus acidoterrestris* (de Carvalho, Vanetti, & Mantovani, 2008). Since bacteria that can readily become resistant to nisin did not become significantly more resistant to bovicin HC5 after they were repeatedly transferred with sublethal doses, it appeared that bovicin HC5 had important characteristics (Mantovani & Russell, 2003).

Inhibition of *L. monocytogenes* by bacteriocins such as bovicin HC5; nisin (Boziaris & Nychas, 2006); curvaticin 13 (Bouttefroy & Milliere, 2000); cerein 8A (Bizani, Morrissy, Dominguez, & Brandelli, 2008); reuterin (Arques et al., 2008; El-Ziney & Jakobsen, 2009); enterocin (Ghrairi, Frere, Berjeaud, & Manai, 2008) and pediocin AcH (Loessner, Guenther, Steffan, & Scherer, 2003) demonstrates that this may be a useful strategy for food processing to ensure microbiological safety.

A combination of preservative methods may work synergistically or at least provide greater protection than a single method alone, thus improving the safety and quality of food (Deegan, Cotter, Hill, & Ross, 2006). Based on this assumption, a combination of bacteriocins has been tested in order to increase antimicrobial activities and improve food safety (Galvez, Abriouel, Lopez, & Ben Omar, 2007).

The objective of the present work was to study the combined effect of bovicin HC5 and nisin on *L. monocytogenes*, *L. innocua* and *S. aureus* in whole milk. Although the effect of nisin combined with other antimicrobial agents has already been extensively studied, this is the first study evaluating the effect of nisin combined with bovicin HC5 on foodborne pathogens.

2. Materials and Methods

2.1 Microorganisms and Growth

S. bovis HC5 was cultured anaerobically as previously described (Mantovani & Russell, 2003). *Lactococcus lactis* ATCC 19435 was cultivated aerobically in de Man, Rogosa and Sharpe (MRS) broth (Merck, Gemany) at 37°C.

L. monocytogenes ATCC 7644, *L. monocytogenes* Scott A, *L. innocua* LMA83 (isolated from a dairy industry), *L. innocua* LMA84 (isolated from Minas cheese), *S. aureus* ATCC 25923, *S. aureus* ATCC 6538 and *S. aureus* Embrapa 4018 (isolated from bovine mastitis) were cultivated in trypticase soy broth (TSB) (Merck, Germany) and incubated at 37°C. The identity of all bacterial strains was confirmed by biochemical tests.

2.2 Preparation and Activity of Bovicin HC5 and Nisin

Extracts of bovicin HC5 were prepared as described by Mantovani et al. (2002). Bovicin HC5 concentration was estimated by serial two-fold dilutions of extract followed by spotting 25 µl on MRS agar using *L. lactis* ATCC 19435 as the indicator organism. Plates were incubated at 37°C for 24 h. One arbitrary unity (AU) was defined as the reciprocal of the highest dilution that showed a zone of inhibition with at least 5 mm diameter.

Nisin solution (Nisaplin®, Danisco, Copenhagen, Denmark) was prepared in phosphate buffer (pH 2.0) and the bacteriocin activity was determined as described earlier.

2.3 Effect of Bovicin HC5 and, or Nisin on the Growth of Listeria *and* S. aureus *in TSB*

To determine the concentration of bovicin HC5 and nisin (individually or combined) that could inhibit *Listeria* and *S. aureus* we performed *in vitro* studies in 96-well microtiter plates. Strains of *L. monocytogenes*, *L. innocua* and *S. aureus* were activated in TSB and incubated at 37°C for 18 h. The cells were harvested by 3000 *g* centrifugation, washed with 0.1% salt peptone water, and then resuspended in TSB. *Listeria* and *S. aureus* cultures (10^6 CFU ml^{-1}) were treated with bovicin HC5 and nisin at concentration of 10, 50, 100 and 150 AU ml^{-1} for the tests with isolated bacteriocins. In the assays using bovicin HC5 and nisin combined, the concentrations varied from 10 to 50 AU ml^{-1}.

The bacteria were incubated at 37°C and the growth was monitored via changes in optical density at 630 nm in an ELISA reader (Thermo Plate, model TP-Reader) for up to 10 h of incubation. Control treatments were performed in TSB inoculated with bacterial cultures without bacteriocins.

2.4 Effect of Bovicin HC5 and, or Nisin on the Growth of Listeria *and* S. aureus *in Whole Milk*

Cultures of *L. monocytogenes* Scott A, *L. innocua* LMA83, and *S. aureus* ATCC 6538 were selected because they presented more resistant after being treated with bacteriocins in TSB. Cells were activated in 5 ml TSB and incubated at 37°C for 18 h, harvested by centrifugation at 3000 *g* for 15 min, washed with 0.1% salt peptone water and then, resuspended in 1 ml of ultra-high temperature (UHT) whole milk to reach 10^7 CFU ml^{-1}. Bovicin HC5 and nisin were added individually (400, 800, and 1200 AU ml^{-1}) or in combination (400, 600, and 800 AU ml^{-1} of each bacteriocin).

The tubes were incubated at 37°C and samples were taken at 0, 3, 6, 9, and 12 h for determination of viable cell number by microdrops (Herbert, 1990) plating aliquots of 25 µl in tryptic soy agar (Oxoid, England). Plates were incubated at 37°C for 12 or 24 h. Control treatments were performed in milk inoculated with bacterial cultures without bacteriocins.

2.5 Statistics

Each experiment was performed at least two times in duplicate. The log of the absorbance and colony forming units was plotted versus time (SigmaPlot version 11.0, USA) and the error bars presented indicate the standard deviation of the mean (shown only in the positive direction).

3. Results

3.1 Effect of Bovicin HC5 and/or Nisin on Listeria *and* S. aureus *in TSB*

Table 1. Effect of bovicin HC5 or nisin on the growth of *Listeria* and *S. aureus*

Microorganism	Bacteriocin Concentration (AU ml^{-1})	Specific growth rate (h^{-1})		Lag phase duration (h)	
		Bovicin HC5	Nisin	Bovicin HC5	Nisin
L. monocytogenes ATCC 7644	0	0.60	0.60	0	0
	10	0.26	-	0	>11
	50	0.21	-	0	>11
	100	-	-	>11	>11
	150	-	-	>11	>11
L. monocytogenes Scott A	0	0.55	0.55	0,5	0
	10	0.32	-	0	9
	50	0.21	-	0	10
	100	-	-	>11	>11
	150	-	-	>11	>11
L. innocua LMA84	0	0.61	0.61	0	0,5
	10	-	-	>11	>11
	50	-	-	>11	>11
	100	-	-	>11	>11
	150	-	-	>11	>11
L. innocua LMA83	0	0.59	0.59	0	0
	10	0.30	0.84	0	8
	50	0.28	-	0	>10
	100	-	-	>11	>11
	150	-	-	>11	>11
S. aureus Embrapa 4018	0	0.84	0.84	0	0
	10	0.82	1.13	8	8
	50	0.75	0.60	8	8
	100	-	-	>11	10
	150	-	-	>11	>11
S. aureus ATCC 25923	0	0.39	0.39	0	0
	10	0.30	0.26	0	2
	50	0.27	-	0	>11
	100	0.28	-	0	>11
	150	0.28	-	0	>11
S. aureus ATCC 6538	0	0.43	0.43	0	0
	10	0.21	0.54	1	6
	50	0.19	0.50	1	7
	100	0.40	0.61	8	8
	150	-	0.57	>11	8

-, no growth; AU, activity units.

The specific growth rate of *L. monocytogenes* ATCC 7644 and *L. monocytogenes* Scott A in TSB was reduced in the presence of bovicin HC5 at concentrations of 10 and 50 AU ml^{-1}, while growth was completely inhibited in the presence of 100 and 150 AU ml^{-1} of this bacteriocin (Table 1). *L. monocytogenes* ATCC 7644 and Scott A were inhibited in TSB even at the smallest nisin concentration of 10 AU ml^{-1} (Table 1). The effect of the bacteriocins bovicin HC5 and nisin was different on *L. innocua*: while *L. innocua* LMA84 was completely inhibited by bovicin HC5 or nisin at 10 AU ml^{-1}, concentrations up to 50 AU ml^{-1} allowed the growth of *L. innocua* LMA83 after a lag phase period (Table 1).

Absorbance reduction of cultures of *L. monocytogenes* and *L. innocua* was observed when both bacteriocins were combined at the concentrations 10 (data not shown) and 50 AU ml^{-1} (Figure 1), suggesting a bactericidal effect. As the behavior pattern was similar for both strains of *L. monocytogenes* and *L. innocua*, only data from *L. monocytogenes* Scott A and *L. innocua* LMA83 are shown in Figure 1.

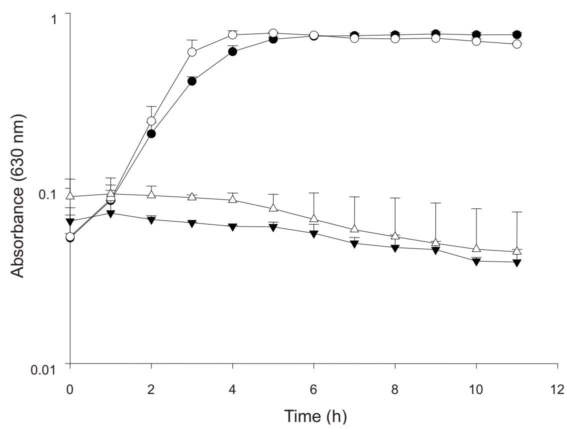

Figure 1. Effect of bovicin HC5 and nisin in combination at 50 AU ml^{-1} of each one on the growth of *L. monocytogenes* Scott A (▼) or *L. innocua* LMA83 (△) in TSB. Control without bacteriocins with *L. monocytogenes* Scott A (●) or *L. innocua* LMA83 (○) is also shown

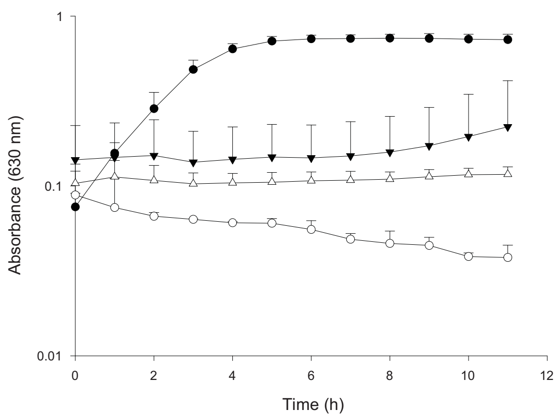

Figure 2. Effect of bovicin HC5 and nisin in combination at 50 AU ml^{-1} of each one on the growth of *S. aureus* Embrapa 4018 (○) or *S. aureus* ATCC 25923 (▼) or *S. aureus* ATCC 6538 (△). Control without bacteriocins with *S. aureus* (●) is also shown

Although the presence of bovicin HC5 or nisin has clearly different impact on the specific growth rate of *S. aureus* Embrapa 4018, ATCC 25923 and ATCC 6538 (Table 1), all three strains were more resistant against the bacteriocins than *Listeria*. Bovicin HC5 at 150 AU ml^{-1} was sufficient to inhibit the growth of two of the three *S. aureus* strains tested.

Bovicin HC5 and nisin combined was more effective than the separate bacteriocins to inhibit the growth of *S. aureus* in TSB. Although the addition of bacteriocins in the combined concentration of 10 AU ml^{-1} (data not shown) has reduced the specific growth rate, inhibition was greater when 50 AU ml^{-1} of each bacteriocin was added (Figure 2). The addition of bovicin HC5 and nisin combined at the concentration of 50 AU ml^{-1}, exerted a greater inhibitory effect on *S. aureus* Embrapa 4018 and ATCC 6538 than the isolated addition of each bacteriocin in the same concentration (Table 1).

3.2 Effect of Bovicin HC5 and/or Nisin on Listeria *and* S. aureus *in Milk*

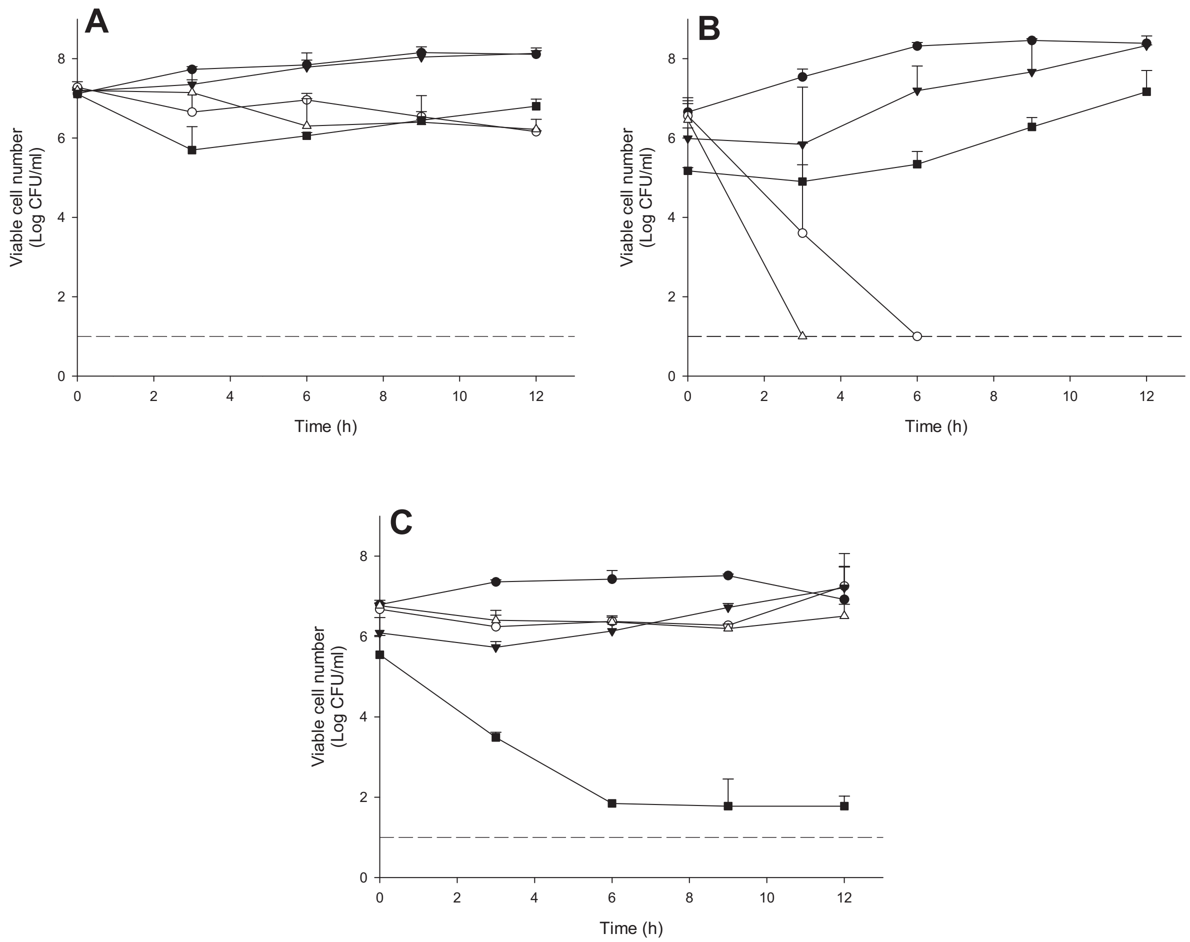

Figure 3. Effect of bovicin HC5 at concentration of 800 AU ml^{-1} (—○—) and 1200 AU ml^{-1} (—△—) or nisin at concentration of 800 AU ml^{-1} (—▼—) and 1200 AU ml^{-1} (—■—) on the growth of *L. monocytogenes* Scott A (**A**), *L. innocua* LMA83 (**B**) and *S. aureus* ATCC 6538 (**C**) in whole milk. Control without bacteriocins is shown (—●—). The dotted line shows the detection limit of the enumeration technique used

L. monocytogenes Scott A, *L. innocua* LMA83 and *S. aureus* ATCC 6538 strains were selected to evaluate the effect of bacteriocins in milk. The addition of bovicin HC5 at 400 (data not shown), 800 and 1200 AU ml^{-1} to milk reduced the viable cell number of *L. monocytogenes* (Figure 3A). Although a pronounced effect was observed when 1200 AU ml^{-1} of nisin was used, the growth was resumed after 3 h of incubation (Figure 3A). The lethal effect of nisin and bovicin HC5 combined on *L. monocytogenes* in whole milk was observed at concentrations of 600 and 800 AU ml^{-1} of each, with a sharp reduction in the cell number to below the detection limit of the technique, which is 10^1 CFU ml^{-1} (Figure 4A).

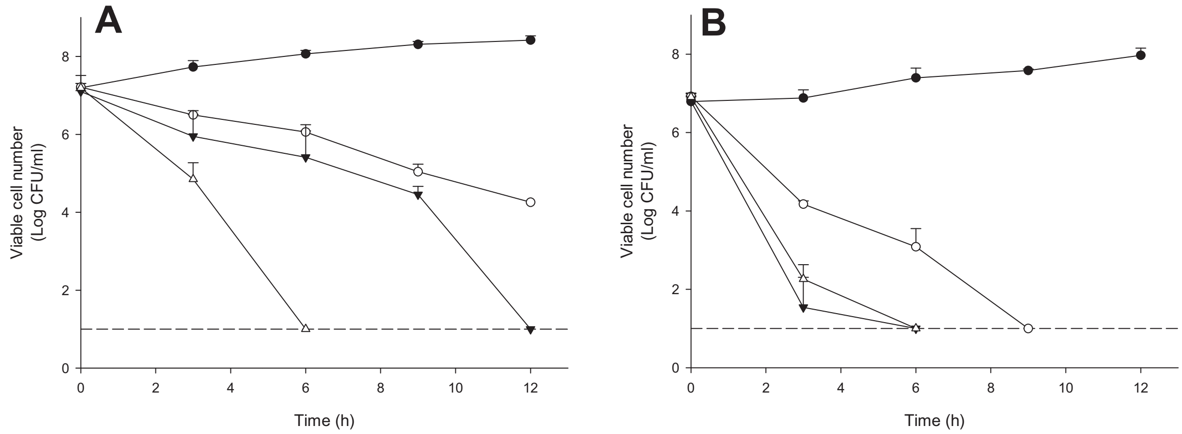

Figure 4. Effect of the combination of bovicin HC5 and nisin at 400 AU ml^{-1} (—○—), 600 AU ml^{-1} (—▼—) or 800 AU ml^{-1} (—△—) of each one on the growth of *L. monocytogenes* Scott A (**A**) and *S. aureus* ATCC 6538 (**B**) in whole milk. Control without bacteriocins is shown (—●—). The dotted line shows the detection limit of the enumeration technique used

Bovicin HC5 concentrations from 400 to 1200 AU ml^{-1} in milk were also bactericidal to *L. innocua* and the viable cell number was reduced by more than 5 log cycles. Although nisin has exerted an inhibitory effect on *L. innocua* by increasing 3 h in the lag phase, the growth was resumed after this period (Figure 3B).

The major resistance of *S. aureus* to the bacteriocins effect observed in TSB was confirmed in milk and the concentration of 800 AU ml^{-1} did not prevent the growth of *S. aureus* ATCC 6538 (Figure 3C). At the highest concentration (1200 AU ml^{-1}), bovicin HC5 exerted a bacteriostatic effect while nisin initially exerted a bactericidal effect during 6 h after exposition followed by a bacteriostatic effect (Figure 3C). However, the combination of bovicin HC5 and nisin reduced the viable cell number of *S. aureus* ATCC 6538 in UHT milk to below the detection limit of the technique, within 9 h of incubation (Figure 4B).

4. Discussion

Bacteriocins have been used as a food preservative, mainly against foodborne pathogens. However, our finding that *Listeria* and *S. aureus* growth can be resumed after the lag phase indicates selection of resistant bacteria to bacteriocins and this is a problem to be addressed before the adoption of a food preservation process. Another study also found a transient antimicrobial effect of 100 AU ml^{-1} of nisin or 320 AU ml^{-1} of curvaticin 13, a bacteriocin produced by *Lactobacillus curvatus* SB13, against *L. monocytogenes* ATCC 7644 being the growth resumed after approximately 9 h of incubation at 37°C (Bouttefroy & Milliere, 2000). *L. monocytogenes* resistant to antimicrobial agents showed an increase in the proportion of saturated fatty acids that can increase the rigidity of the cell membrane, making it less fluid and thus preventing the penetration of molecules of bacteriocins (Naghmouchi, Belguesmia, Baah, Teather, & Drider, 2011; Naghmouchi, Kheadr, Lacroix, & Fliss, 2007). The resistance of some species of *Staphylococcus* to nisin is associated with increased amounts of D-alanine in the structure of teichoic acids present in the peptidoglycan layer of these bacteria becoming the cells more positively charged (Peschel et al., 1999). These changes may hinder the interaction of nisin, positively charged, with the surface of cells.

The antimicrobial activity of nisin is related to its abilities to bind specifically to lipid II, a precursor in the biosynthesis of cell wall, and to form pores leading to cell death due to loss of intracellular compounds (Breukink & de Kruijff, 2006; Breukink et al., 2003). As a lantibiotic, bovicin HC5 has a primary mode of action similar to nisin, which involves specific interaction with lipid II. However, some differences regarding the pore-forming capacity of both bacteriocins were observed in model membranes: the pore-formation by bovicin HC5 was clearly dependent on membrane thickness, being observed only in thinner membranes (Paiva, Breukink, & Mantovani, 2011). Independent on the pore formation, bovicin HC5 maintains its antibacterial activity by recruiting lipid II molecules in a prepore-like structure, and consequently preventing the use of such molecules in the cell wall synthesis (Paiva et al., 2011).

Another issue to consider is the variation of resistance to bacteriocins found among strains of the same species. For example, we noted that bovicin HC5 and nisin have exerted an inhibitory effect on *L. monocytogenes* but strain ATCC 7644 was more sensitive to nisin while *L. monocytogenes* Scott A was more sensitive to bovicin HC5. The sensitivity variation to nisin among different strains of *S. aureus* isolated from dairy products was also observed (Sudagidan & Yemenicioglu, 2012). Mantovani and Russell (2003) found that some bacteria resistant to nisin showed no significant resistance to bovicin HC5, even after repeated treatments with sublethal doses of that bacteriocin. Higher concentrations of bacteriocins could increase the bactericidal effect and reduce the chances of selecting resistant bacteria and to have an inhibitory effect on several strains that could contaminate food.

Another alternative to reduce the selection of resistant cells would be the combined use of bacteriocins. In this study we showed that the impact of bovicin HC5 and nisin can be greatly enhanced if both bacteriocins are applied in combination. In fact, concentrations as low as 10 AU ml^{-1} of each bacteriocin in TSB resulted in reduction of the absorbance of cultures of *L. monocytogenes* Scott A and *L. innocua* LMA83 indicating cell lysis. The results showed that the combined effect of bovicin HC5 and nisin allow the use of lower dosages compared to the individual application.

According to Gálvez et al. (2007) when cells are exposed to a combination of antimicrobial factors, the intensity of the damage can be increased since these factors may act on different sites of the same target. The repair of multiple damages may require a high energy expenditure, resulting in energy depletion and cell death. The addition of nisin (0.5% w/v) in ready to eat salad did not achieved the complete inactivation of *L. monocytogenes* but the combination of this bacteriocin with enterocin AS-48 reduced the concentration of viable cells below the detection limit of the technique after 24 h of incubation (Molinos et al., 2009).

Although the addition of bacteriocins in milk used for consumption is not permitted, this product serves as an important system to evaluate the influence of chemical composition of milk on the activity of bacteriocins. Milk is a complex mixture of substances such as water, protein, lactose and fat, which can affect the effectiveness of bacteriocins. The addition of nisin at concentrations of 62.5, 125, 250 and 500 IU ml^{-1} in skim milk had a significant effect on *L. monocytogenes*, while in raw milk, the inhibitory activity was only moderate, with a rapid reduction of the *L. monocytogenes* population during two days of incubation before growth was resumed (Kim, Choi, Bajpai, & Kang, 2008). These results demonstrate that the activity of bacteriocin is dependent on the fat content in milk, and the interaction between the lipids of milk and nisin may limit the application of this bacteriocin in dairy fat products (Sobrino-Lopez & Martin-Belloso, 2008).

No research to assess the synergistic effect of bovicin HC5 with another antimicrobial agent in milk was carried out so far. However, results from the synergistic effect of nisin and other antimicrobial substances can be exemplified. Nisin combined with garlic extract showed potential antilisterial activity with a synergistic effect in reducing the viable cell number of *L. monocytogenes* in milk and skim milk in 14 days (Kim et al., 2008). Synergistic effect was also found by Arqués et al. (2008) evaluating the combination of nisin and reuterin, antimicrobial produced by *Lactobacillus reuteri*, in milk. These authors reported a reduction of approximately two log cycles of *S. aureus* treated with these antimicrobials in combination after 24 h of incubation.

5. Conclusions

The present study demonstrates that the combination of bovicin HC5 and nisin against *Listeria* and *S. aureus* in TSB and milk was more effective than the addition of these bacteriocins individually. These results indicate their potential use as biopreservatives in food. However, the molecular basis of synergistic effect between bovicin HC5 and nisin are now under investigation. Furthermore, the combination of bovicin HC5 with others antimicrobial agents against foodborne pathogens is focus of further research.

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Influence of Tyre Inflation Pressure and Wheel Load on the Traction Performance of a 65 kW MFWD Tractor on a Cohesive Soil

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Abstract

The choice of tractor configuration is of primary importance in tillage operations for the optimisation of traction performance, i.e. for limiting slip which involves energy loss. To a great extent, this aspect affects the fuel consumption and the time required for soil tillage. Tyre inflation pressure and wheel load are both easily managed parameters which play a significant role in controlling the traction performance of a tractor. The present study aimed to investigate the influence of tyre inflation pressure and wheel load on the traction performance of a mechanical front wheel drive MFWD tractor (65 kW engine power) on an agricultural clay (C) Vertic Cambisol on the basis of results of traction tests and simulations with a semi-empirical soil-tyre interaction model adapted for MFWD vehicles. The traction tests were carried out using four tractor configurations with two tractor weights (40.8 kN and 50.2 kN) and two tyre inflation pressures (60 kPa and 160 kPa). Traction performance was considered in terms of drawbar pull, traction coefficient, tractive efficiency, power delivery efficiency and specific fuel consumption in relation to wheel slip. A decrease in tyre pressure and an increase in wheel load resulted in higher drawbar pull however, only the former produced improvements in terms of coefficient of traction, tractive efficiency, power delivery efficiency and specific fuel consumption, while the only significant benefit resulting from the latter was a reduction in specific fuel consumption at a tyre pressure of 160 kPa and a slip of under 15%.

Keywords: soil-tyre interaction, traction performance, tractive efficiency, power delivery efficiency, specific fuel consumption

Nomenclature

a	Parameter of the parabolic equation which defines the shape of the contact surface (m^{-1})
A	Fitting parameter for the power delivery efficiency as a function of wheel slip
B	Fitting parameter for the specific fuel consumption as a function of wheel slip (kW h g^{-1})
b	Contact patch smaller dimension (width of the tyre) (m)
c	Soil cohesion (kPa)
ds	Infinitesimal area of the soil-tyre contact surface (m^2)
DP	Drawbar pull (kN)
D_{rim}	Rim diameter (m)
e_t	Eccentricity of the vertical soil reaction relative to the rear point of the contact surface (m)
e_0	Eccentricity of the centre of the wheel relative to the rear point of the contact surface (m)
FC	Gravimetric fuel consumption (kg h^{-1})
GT	Gross traction (kN)
h	Height of the drawbar (m)
i	Wheel slip
j	Soil shear displacement (m)
k	Soil shear deformation modulus (m)
$K_{c,f}$	Soil cohesive modulus of deformation for the front wheel (kN $m^{-(n+1)}$)
$K_{c,r}$	Soil cohesive modulus of deformation for the rear wheel (kN $m^{-(n+1)}$)
K_{carc}	Tyre carcass stiffness (kN m^{-1})
K_s	Theoretical speed ratio

K_v	Coefficient of vertical stiffness of the tyre for unit length of the contact surface (kN m^{-2})
$K_{\varphi,f}$	Soil frictional modulus of deformation for the front wheel (kN m$^{-(n+2)}$)
$K_{\varphi,r}$	Soil frictional modulus of deformation for the rear wheel (kN m$^{-(n+2)}$)
l_A	Fitting parameter for the power delivery efficiency as a function of wheel slip
l_B	Fitting parameter for the specific fuel consumption as a function of wheel slip
L	Wheelbase of the tractor (m)
M_{GT}	Driving torque relative to the gross traction (kN m)
M_r	Resistance moment due to eccentricity of the vertical soil reaction respect to the wheel centre (kN m)
n_f	Exponent of soil deformation for the front wheel
n_r	Exponent of soil deformation for the rear wheel
NT	Net traction (kN)
p_h	Horizontal component of the elementary force at soil-tyre contact surface (kN)
p_s	Vertical soil pressure (kPa)
p_v	Vertical component of the elementary force at soil-tyre contact surface (kN)
P_{in}	Tyre inflation pressure (kPa)
PTO	Power-take-off (kW)
R	Unloaded radius of the wheel (m)
R_c	Soil compaction resistance (kN)
R_{GT}	Distance from the centre of the wheel to the point of application of the gross traction (m)
R_r	Rolling radius of the wheel (m)
SFC	Specific fuel consumption (kg kW^{-1} h^{-1})
T	Total driving torque on the wheel (kN m)
V_a	Actual forward velocity of the wheel (m s^{-1})
W	Vertical component of the total soil reaction / wheel load (kN)
$W_{tractor}$	Tractor weight (kN)
W_0	Wheel load in stationary condition (kN)
x	Horizontal coordinate (m)
x_0	Length of the soil-tyre contact surface in the horizontal direction (m)
z	Vertical coordinate (soil sinkage) (m)
z_0	Rut depth (m)
Greek letters	
α	Angle between the tangent to the infinitesimal area of the soil-tyre contact and the *x*-axis (°)
δ	Tyre vertical deflection (m)
ΔK_p	Inflation pressure dependence of the tyre (kN m^{-1} kPa^{-1})
η_{PD}	Power delivery efficiency
η_{tr}	Tractive efficiency
θ_A	Fitting parameter for the power delivery efficiency as a function of wheel slip
θ_B	Fitting parameter for the specific fuel consumption as a function of wheel slip
μ_{tr}	Traction coefficient
σ	Normal stress at soil-tyre contact surface (kPa)
σ_v	Vertical component of the surface traction (kPa)
τ	Shear stress at soil-tyre contact surface (kPa)
τ_{max}	Soil strength (kPa)
φ	Angle of soil shear resistance (°)
ω	Angular velocity of the wheel (s^{-1})

1. Introduction

Since the traction performance of a tractor has a major impact on both fuel consumption and the time required for soil tillage, optimising this performance is clearly of crucial importance in tillage management.

Traction performance is usually expressed in terms of the pulling force available at the tractor drawbar (drawbar pull), or alternatively in terms of the traction coefficient μ_{tr}, which is defined as the drawbar pull DP to tractor weight $W_{tractor}$ ratio:

$$\mu_{tr} = \frac{DP}{W_{tractor}} \tag{1}$$

Other important parameters involving the energy aspects of traction performance are tractive efficiency η_{tr} and power delivery efficiency η_{PD}. The tractive efficiency of a drive wheel is defined as:

$$\eta_{tr} = \frac{NT\,V_a}{T\omega} \tag{2}$$

which expresses the ratio of output power to input power of the wheel, NT being the net traction force, V_a the actual forward velocity of the wheel, T the total driving torque on the wheel, and ω the angular velocity of the wheel. The same tractive efficiency can be defined for the tractor as:

$$\eta_{tr} = \frac{DP\,V_a}{\sum T\omega} \tag{3}$$

and represents the fraction of power delivered to the tractor wheels that is available as drawbar power. Servadio (2010) considered both the traction coefficient and the tractive efficiency to characterise field performance of several wheeled and tracked vehicles in central Italy.

Power delivery efficiency is defined as the ratio of delivered tractive power (drawbar power) to tractor input power from the engine, and represents the fraction of power produced by the engine of a tractor that is available as tractive power (Shell, Zoz, & Turner, 1997; Turner, Shell, & Zoz, 1997). In order to consider the engine power input, the equivalent *PTO* (power-take-off) power can be used (Zoz, Turner, & Shell, 2002), in which case the power delivery efficiency can be defined as:

$$\eta_{PD} = \frac{DP\,V_a}{Equivalent\ PTO\ power} \tag{4}$$

In terms of fuel consumption, a parameter related to the traction performance of the tractor is the specific fuel consumption *SFC*, defined as the ratio of the fuel consumption expressed in kg h^{-1} (gravimetric) or l h^{-1} (volumetric) to the engine power input, or alternatively to the drawbar power.

The traction performance of a wheeled tractor is the result of a stress-strain interaction between the tractor wheels and the topsoil. This interaction is affected by several factors, including the mechanical behaviour of the topsoil, power and geometry (wheelbase and drawbar height) of the tractor, number of drive wheels, wheel load, wheel slip, tyre dimensions (width and diameter), tyre inflation pressure and stiffness, all of which exert a significant influence. While most of the above factors are more or less constrained, wheel load and tyre inflation pressure can be varied within wide ranges, allowing easy management of the traction performance of the tractor. Consequently, these factors are highly advantageous for practical applications. The influence of wheel load and tyre inflation pressure on tractor traction performance has been investigated using both a theoretical and an experimental approach.

With regard to the former approach, the semi-empirical models of interaction between soil and a pneumatic wheel based on Bekker's theory (Bekker, 1960) offer a valid framework for the better understanding and simulation of the effects of both tyre inflation pressure and wheel load on the traction performance of the tractor-soil system. In this context several approaches have been presented assuming the contact surface between soil and tyre to be a combination of a flattened portion and the unloaded contour (Bekker, 1960; Wong, 1989), or as an arc of an equivalent rigid wheel of larger diameter (Fujimoto, 1977), or also described as a parabolic configuration with its apex at the front point of contact (Schmid, 1995). More recently, Shmulevich and Osetinsky proposed a model based on a parabolic soil-tyre contact surface with its apex at the rear point of contact (Shmulevich & Osetinsky, 2003; Osetinsky & Shmulevich, 2004), which presents a simple mathematical treatment and allows a reliable simulation of traction performance.

With regard to the latter approach, many authors have reported experimental results showing some benefits of reduced tyre inflation pressure for tractor traction performance (Zombori, 1967; Gee-Clough, McAllister, & Evernden, 1977; Burt & Bailey, 1982; Turner, 1993; Zoz & Grisso, 2003). Whilst evident for radial-ply tyres, these benefits in some cases turned out to be less or not at all significant for bias-ply tyres (Lee & Kim, 1997). Serrano, Peça, Silva, and Márquez (2009) studied the performance of a tractor (59 kW engine power) with two static ballasts, with and without liquid tyre ballast, and at three different inflation pressures. The use of liquid ballast in the tyres turned out not to improve work-rate and besides to increase fuel consumption per hectare of 5-10%. The use of higher tyre inflation pressures produced a slight reduction in work-rate (3-5%) with a large increase in fuel consumption per hectare (10-25%). Burt, Balley, Patterson, & Taylor (1979) investigated the influence of dynamic wheel load on tractive efficiency on both a compacted and an uncompacted soil, observing that, with constant travel reduction (slip), an increase in dynamic load produced in the former case an increase and in the latter case a decrease in tractive efficiency. Charles (1984) carried out tractor-traction tests on a low-plasticity silt soil in both a tilled (soft) and firm condition at different static loads and tyre inflation pressures. His findings show that both an increase in static load and a decrease in tyre pressure resulted in higher traction performance in terms of drawbar pull and tractive efficiency for both of the soil conditions considered. Lyne, Burt, and Meiring (1984) reported results of traction tests with a 4WD tractor on a Westleigh clay in two soil conditions and with several combinations of static load and tyre pressure, showing that as static load increased at each inflation pressure, so did drawbar pull, drawbar power and power demand on the engine, with a corresponding decrease in specific fuel consumption (drawbar power basis). According to results reported by Turner (1993) and Zoz and Grisso (2003), an increase in tractor weight (wheel load), obtained with ballasts and tyre inflation pressure adapted to the weight, makes for higher drawbar pull, although it does not seem to result in a significant variation in terms of traction coefficient or power delivery efficiency. Results of traction tests reported by Zoz and Grisso (2003) for a single 520/85R46 radial tyre with inflation pressure adapted for different wheel loads showed a negligible influence on maximum tractive efficiency. Burt, Lyne, Meiring, and Keen (1983) and Burt and Bailey (1982) showed how, for a given drawbar pull, the tractive efficiency of both radial-ply and bias-ply tyres can be maximised by selecting proper levels of dynamic load and inflation pressure. Lyne et al. (1984) also pointed out the importance of an appropriate choice of both dynamic load and tyre inflation pressure in order to optimise the tractive efficiency of a tractor. Moreover, it was observed that operating at optimum tractive efficiency allows minimum specific fuel consumption (Lyne et al., 1984; Jenane, Bashford, & Monroe, 1996). Gee-Clough, Pearson, and McAllister (1982) demonstrated the key role of a wheel load properly matched to tractor power, speed, and drawbar pull at low tyre inflation pressure (110 kPa or less), in the optimisation of the power output of wheeled tractors in frictional-cohesive soils. This variety of studies has produced results which in some cases appear to contradict one another. It should be pointed out, however, that the widely differing experimental conditions considered (soil and tyre types, wheel load range, tyre pressure range) make it difficult to draw proper comparisons, as do the different layouts and methodologies used for the traction tests.

In this context, the issue of improving traction performance of a tractor by ballasting or by reducing the inflation pressure of the tyres is thought to require further and deeper understanding in order to better define clear indications for a correct choice of the tractor configuration, this latter considerably contributing in more appropriate tillage management. Furthermore, some of the studies presented either dealt with big tractors (Turner, 1993; Zoz & Grisso, 2003) or the single wheel testers (Burt et al., 1979; Burt & Bailey, 1982; Gee-Clough et al., 1977), whilst the performance of medium powered tractors, which are reasonably widespread in Central Europe, has received less attention.

In this paper, the influence of tyre inflation pressure and wheel load on the traction performance of a MFWD tractor (65 kW engine power) on an agricultural clay (C) Vertic Cambisol is compared and discussed on the basis of results of field traction tests as well as simulations with a semi-empirical soil-tyre interaction model adapted for MFWD vehicles, with a mechanistic interpretation of results. In addition, two equations describing the relationships power delivery efficiency-wheel slip and specific fuel consumption-wheel slip are presented.

2. Materials and Methods

2.1 Soil-Tyre Interaction Modelling

Forces acting on the driven pneumatic wheel with a detail of the elementary forces acting at soil-tyre contact according to Shmulevich and Osetinsky (2003) are shown in Figure 1.

The following assumptions are considered: the soil behaves as a plastic non-linear medium, the wheel rolls in steady-state motion at a low velocity, tyre deformations are linear elastic, the wheel-soil interaction is two dimensional (plane-strain approach). This latter assumption implies that the rut depth is the same across the width, and the width is the same along the contact surface, moreover all values are referred to the unit width of the wheel.

According to Bekker's theory, the vertical soil pressure along the soil-tyre contact surface is assumed to be equal to the soil pressure beneath the compression plate of a bevameter at the same depth:

$$p_s = \left(\frac{K_c}{b} + K_\varphi\right) z^n \tag{5}$$

wherein p_s is the vertical soil pressure under the plate, z is the soil sinkage, n is the exponent of deformation, and b is the smaller dimension of the contact patch (width of the tyre), whilst K_c and K_φ are the cohesive and frictional modulus of soil deformation, respectively.

The vertical component of the total soil reaction must balance out the wheel load W, this condition is expressed as follows:

$$W = \int_0^{x_0} p_s b dx \tag{6}$$

wherein x_0 is the maximum value of the horizontal coordinate according to Figure 1.

The equation of the parabolic contact surface (Osetinsky & Shmulevich, 2004) expresses the sinkage z as a function of the horizontal coordinate x:

$$z = z_0 - ax^2 \tag{7}$$

z_0 being the rut depth and a being the parameter of the parabolic equation.

Integral 6 is solved by means of a series expansion of function 7 limited to the second term, similarly to the approach reported by Wong (2008). This results in:

$$W = \frac{K_c + bK_\varphi}{3} z_0^n \sqrt{\frac{z_0}{a}}(3-n) \tag{8}$$

Tyre stiffness is defined according to Lines and Murphy (1991) as the sum of two components, the carcass stiffness K_{carc} and the product $\Delta K_p P_{in}$, where ΔK_p is the inflation pressure dependence of the tyre and P_{in} is the inflation pressure. Consequently, the stiffness has both a constant component and a component which varies with the inflation pressure.

The same equilibrium condition in equation 6 can be expressed in terms of the tyre stress state according to its stress-deflection relationship:

$$W = K_v\left\{\frac{ax_0^3}{3} - \sqrt{R^2 - e_0^2}\left(x_0 - \frac{e_0}{2}\right) + \frac{x_0 - e_0}{2}\sqrt{R^2 - (x_0 - e_0)^2} + \frac{R^2}{2}\left[arcsin\left(\frac{x_0 - e_0}{R}\right) + arcsin\left(\frac{e_0}{R}\right)\right]\right\} \tag{9}$$

where e_0 is the eccentricity of the centre of the wheel relative to the rear point of the contact surface (Figure 1), R is the unloaded radius of the wheel, and K_v is the coefficient of vertical stiffness of the tyre for unit length of contact surface, which can be expressed as a function of K_{carc} and the product $\Delta K_p P_{in}$:

$$K_v = \frac{W}{R^2 \, arcsin\left(\frac{x_0}{2R}\right) - \frac{x_0}{2}\left(R - \frac{W}{K_{carc} + \Delta K_p P_{in}}\right)} \tag{10}$$

A detailed derivation of equation 9 is described in Osetinsky and Shmulevich (2004).

Rut depth z_0 and the parameter a which define the soil-tyre contact surface are determined by simultaneously solving equations 8 and 9.

The horizontal component of the elementary force p_h and the vertical component of the elementary force p_v acting at soil-tyre contact are given by:

$$p_h = (\sigma ds) sin\alpha - (\tau ds) cos\alpha \tag{11}$$

$$p_v = (\sigma ds) cos\alpha + (\tau ds) sin\alpha \tag{12}$$

where ds is the infinitesimal area of the contact surface and α is the angle between the tangent to the infinitesimal area of the soil-tyre contact surface and the x-axis (Figure 1), whilst σ and τ are the normal stress and the tangential stress at the soil-tyre contact surface, respectively. Moreover, it turns out that (Figure 1):

$$ds \, sin\,\alpha = bdz \tag{13}$$

and

$$ds \, cos\,\alpha = bdx \tag{14}$$

The shear stress τ at each point of the contact surface depends on the normal stress σ, the soil cohesion c, the angle of soil shear resistance φ, the soil shear deformation modulus k, and the soil shear displacement j along the contact surface. This dependence is described by the well known equation proposed by Janosi and Hanamoto (1961):

$$\tau = \left(c + \sigma \tan\varphi\right)\left(1 - e^{-j/k}\right) \tag{15}$$

The soil shear displacement at each point of the contact surface is calculated by integrating the component of the absolute velocity tangent to the surface (slip velocity) over time, similarly to the approach described by Wong and Reece (1967) for a rigid wheel. The calculation of the soil shear displacement is described in detail in Osetinsky and Shmulevich (2004) and yields the following integral to be solved by a numerical approach:

$$j = \int_{x}^{x_0} \frac{\left[ax\left(x - 2e_0\right) + \sqrt{R^2 - e_0^2} - \frac{V_a}{\omega}\right]\left[ax\left(x - 2e_0\right) + \sqrt{R^2 - e_0^2}\right]}{\sqrt{1 + \left(2ax\right)^2}\left[\left(ax^2 - \sqrt{R^2 - e_0^2}\right)^2 + \left(x - e_0\right)^2\right]} dx \tag{16}$$

In integral 16, V_a is the actual forward velocity of the wheel, whilst ω is the angular velocity of the wheel. The wheel slip i relates the actual forward velocity and the angular velocity of the wheel:

$$i = \frac{\varpi R_r - V_a}{\varpi R_r} \tag{17}$$

where R_r is the rolling radius of the wheel.

The normal stress at each point of the contact surface is given by:

$$\sigma = \frac{\sigma_v - c\left(1 - e^{-j/k}\right)2ax}{1 + tg\varphi\left(1 - e^{-j/k}\right)2ax} \tag{18}$$

wherein σ_v is the vertical component of the surface traction, defined as:

$$\sigma_v = \frac{p_v}{bdx} = \frac{K_v \delta}{b} \tag{19}$$

where δ is the vertical tyre deflection at each point of the contact surface (Figure 1) which can be defined on the basis of the geometry of the contact surface as:

$$\delta = \sqrt{R^2 - \left(x - e_0\right)^2} - \sqrt{R^2 - e_0^2} + ax^2 \tag{20}$$

The gross traction GT is obtained by using a numerical approach to integrate the horizontal components p_h of the elementary force over the contact surface. According to equations 11, 13, 14, 15 and 18 this condition is defined as:

$$GT = \int_{x}^{x_0} \frac{\sigma_v\left[2ax - \tan\varphi\left(1 - e^{-j/k}\right)\right] - c\left(1 - e^{-j/k}\right)\left[\left(2ax\right)^2 + 1\right]}{1 + 2ax\left(1 - e^{-j/k}\right)\tan\varphi} bdx \tag{21}$$

The main resisting forces acting on a tractor moving on a flat terrain are represented by the internal resistance of the running gear and the resistance due to the interaction with the terrain. Since the latter factor is, to a great extent, the most significant (Bekker, 1960; Wong, 2008), the internal resistance can be neglected, at least in the first approximation. The resistance due to interaction with the terrain, according to Figure 1, corresponds to the soil compaction resistance R_c. Additional soil bulldozing resistance must be taken into account in soft soils where wheel sinkage is significant. In the cases considered here, the bulldozing effect may be reliably expected not to occur because of the limited wheel sinkage values. The soil compaction resistance is calculated as the vertical work performed in making a rut of a depth z_0 (Bekker, 1960):

$$R_c = \left(K_c + bK_\varphi\right)\frac{z_0^{n+1}}{n+1} \tag{22}$$

The net traction NT is finally calculated as:

$$NT = GT - R_c \tag{23}$$

According to Figure 1, the total driving torque T on the wheel is calculated as:

$$T = M_{GT} + M_r \tag{24}$$

The term M_{GT} is the driving torque relative to the gross traction GT, which is given by:

$$M_{GT} = GT\ R_{GT} \tag{25}$$

where R_{GT} is the distance from the centre of the wheel to the point of application of the gross traction (Figure 1), calculated as described in Osetinsky and Shmulevich (2004).

The term M_r is the resistance moment due to the eccentricity of the vertical soil reaction with respect to the wheel centre on which the wheel load is applied (Figure 1):

$$M_r = W(e_t - e_0) \tag{26}$$

The eccentricity relative to the rear point of the soil-tyre contact surface e_t is calculated from the equilibrium of the moving wheel, according to the original model (Osetinsky & Shmulevich, 2004).

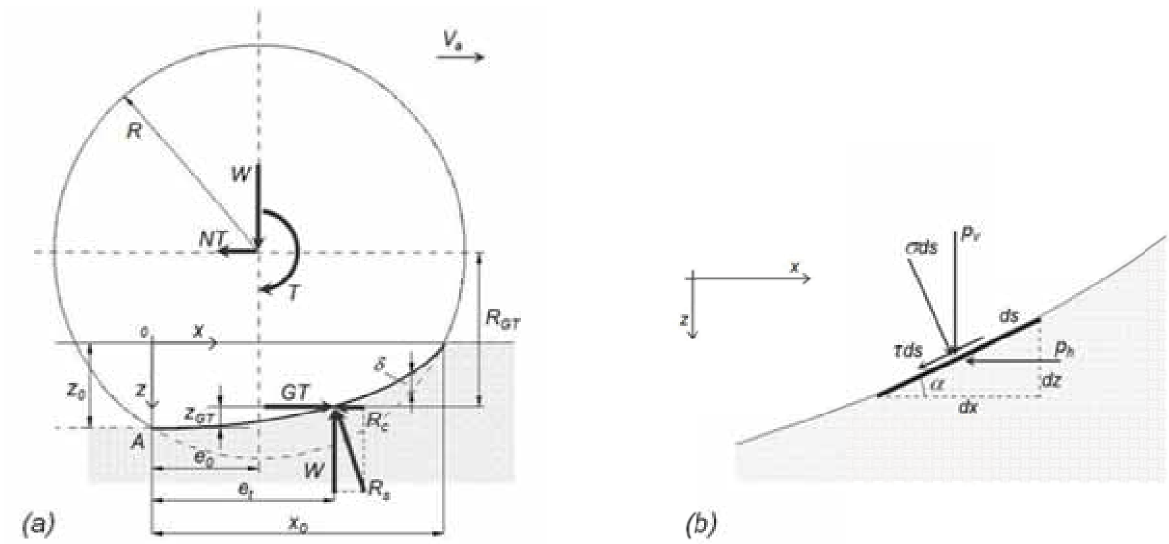

Figure 1. Interaction between soil and a driven pneumatic wheel *(a)*, with the detail of the elementary forces acting at the soil-tyre contact surface *(b)*, according to Shmulevich and Osetinsky (2003)

2.2 Traction Performance of a MFWD Tractor Modelling

The soil-tyre interaction model was adapted to a MFWD tractor by considering the multipass effect, the dynamic wheel load due to the load transfer effect and the theoretical speed ratio K_s which controls the ratio between the slip of the front wheel i_{front} and the rear wheel i_{rear} of a MFWD tractor in straight line motion with rigid coupling between the front and the rear axles.

The multipass effect was considered by means of a differentiated mechanical characterisation of soil interacting with the front and rear wheel, respectively. Soil parameters were derived with bevameter tests before tractor passage as well as on the rut left from the passage of the front wheel, according to Bekker (1960).

When the net traction force is developed, the distribution of the tractor load between the front and rear axles differs from that of the stationary state owing to the transfer of load towards the rear axle. Such an effect is referred to as the load transfer effect, and causes the rear axle load to rise when net traction increases, with an opposite effect on the front axle. The dynamic wheel load was determined on the basis of the equilibrium condition of the tractor body (Figure 2), as follows:

$$W_f = W_{0,f} - \Delta W \tag{27}$$

for the front wheel, and

$$W_r = W_{0,r} + \Delta W \tag{28}$$

for the rear wheel.

The terms $W_{0,f}$ and $W_{0,r}$ represent the stationary wheel loads on the front and rear wheel, respectively, whilst W_f and W_r are the wheel loads in dynamic conditions on the front and rear wheel, respectively. The term ΔW represents the difference between the wheel load in a stationary and a dynamic condition owing to the load transfer effect. According to Figure 2 ΔW is calculated as:

$$\Delta W = \frac{T_f + T_r + \left(NT_f + NT_r\right)\left(h - R_{r,r}\right) + NT_f\left(R_{r,r} - R_{r,f}\right)}{L} \tag{29}$$

wherein T_f, NT_f, $R_{r,f}$ and T_r, NT_r, $R_{r,r}$ are, in order, the total driving torque, net traction and rolling radius of the front and rear wheel respectively; h is the height of the drawbar measured on the field in the operating configuration, and L is the wheelbase of the tractor.

Equation 29 is derived by assuming that the rolling radius is a good approximation of the height of the wheel hub and is constant, and the rut depth is small enough to be neglected in the calculation. The system of Equations 27, 28 and 29 is valid when the pulling force is applied horizontally, which means that the total tractor weight remains constant, with only its distribution changing between the front and rear axles.

For a MFWD tractor with rigid coupling between the front and the rear axles, the ratio of the theoretical speed of the front wheel to that of the rear wheel K_s is fixed, and hence there is a precise relationship between the slip of the front wheel i_{front} and that of the rear wheel i_{rear} in straight line motion:

$$i_{front} = 1 - \frac{\left(1 - i_{rear}\right)}{K_s} \tag{30}$$

Preliminary tests with the MFWD 65 kW tractor in the four configurations considered have indicated values of K_s very close to 1 with a minimum of 0.993 and a maximum of 1.002, allowing a simplified analysis in which the slip of the front and rear wheels are assumed to be the same.

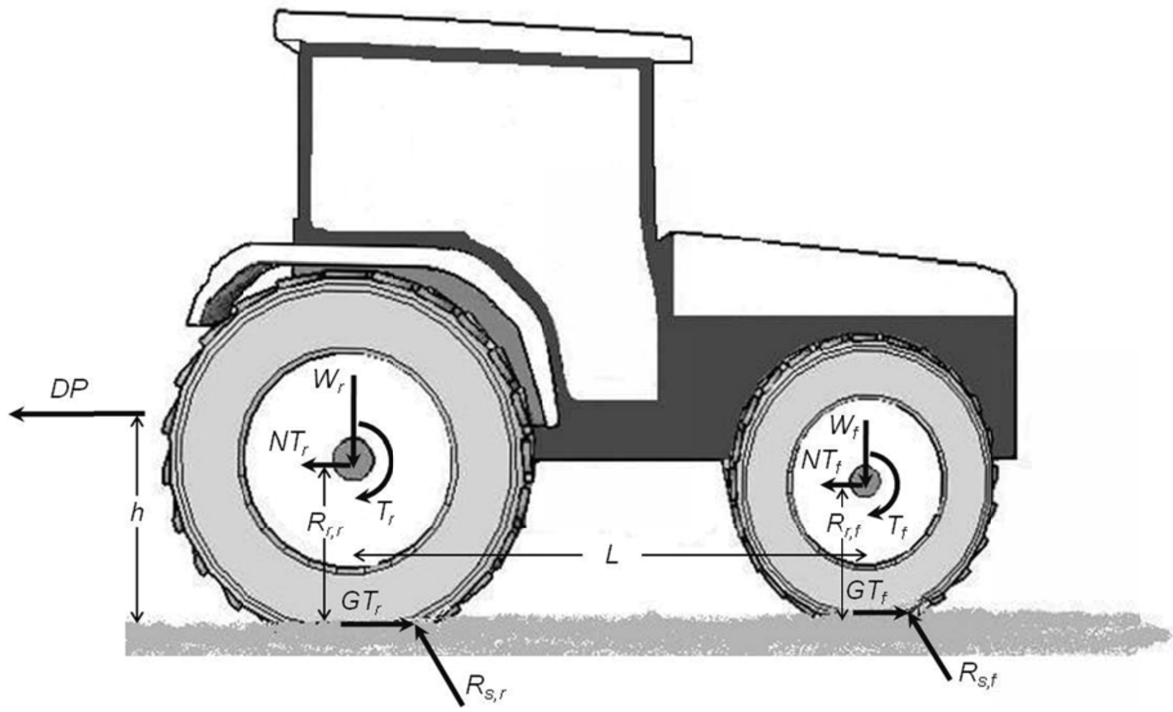

Figure 2. Forces on a MFWD tractor

The model described was written in a Visual Basic code. The main steps in the calculation of traction performance are reported in the flowchart in Figure 3. The calculation procedure can be divided into two main steps: the first defines the soil-tyre contact surface and the second calculates the traction performance in terms of gross traction, soil compaction resistance, net traction, and total driving torque. Since a variation in slip in the range considered causes the tractor weight to be distributed differently between the front and the rear axles, the calculation is repeated until a defined $slip_f$ value is reached. The traction performance of the tractor-soil system is given by the sum of the traction performances obtained for the two axles.

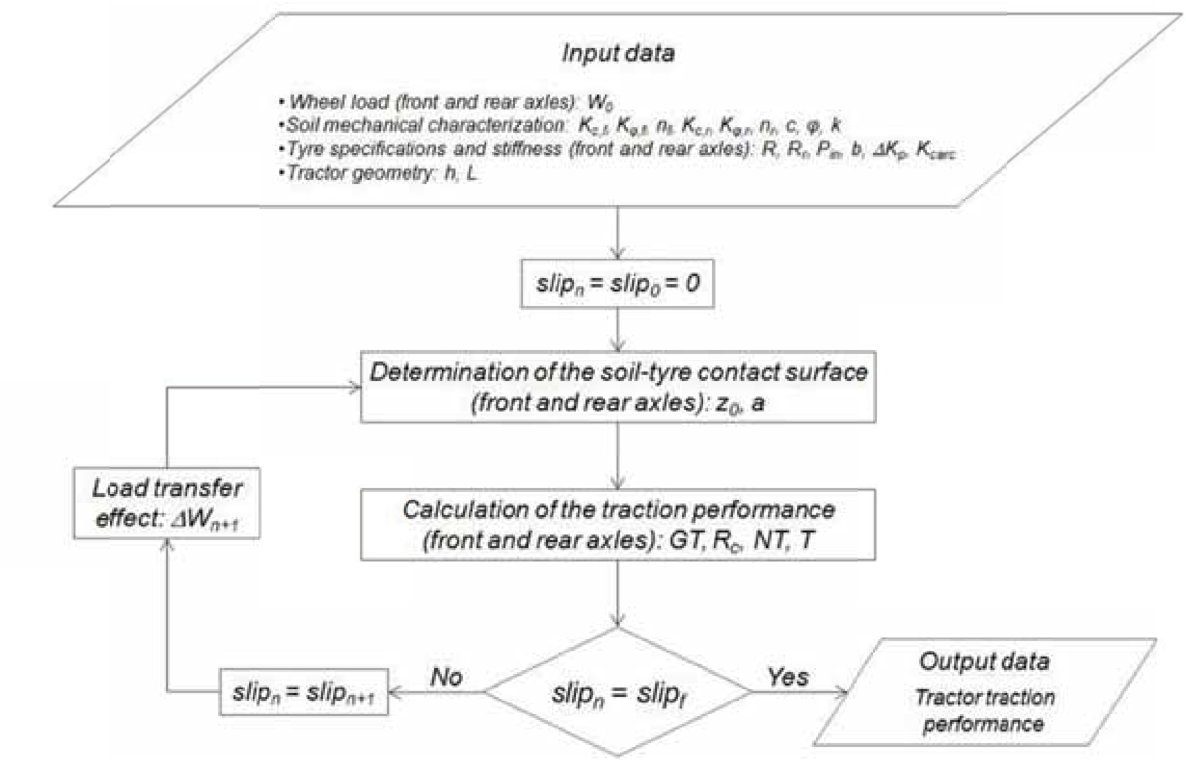

Figure 3. Flowchart of the calculation procedure for tractor traction performance

2.3 Design of Traction Tests

The traction tests took place in a flat agricultural field of clay (C) Vertic Cambisol in Ettenhausen, Switzerland [47°28’52”N, 8°54’14”E].

Corridors 4 m wide and 70 m long were marked out in the field. The corridors were navigated in steady-state motion, controlling the drawbar pull developed by the pulling tractor with a braking tractor used as a dynamometer. Drawbar pull was varied from one corridor to the next, as was, therefore, the slip, which ranged between 6% and 28%. The layout of the traction test in steady-state motion along a corridor is sketched in Figure 4.

A 200 kN load cell (HBM U2B, Darmstadt, Germany) was used to measure drawbar pull. Actual forward velocity was measured by a radar velocity sensor (DICKEY-john RVS2, Auburn, Illinois, USA), whilst the wheel rolling velocity was recorded via a wireless wheel speed sensor (two pulses per wheel revolution) set on a rear wheel of the pulling tractor. An automatic acquisition system in the braking tractor recorded and displayed all these parameters.

In order to measure fuel consumption, the pulling tractor was fitted with an external fuel tank and a switch for changing between the internal and external fuel tanks. Fuel consumption was measured as the difference in weight of the external fuel tank before and after driving along a corridor.

The pulling tractor moved in a rectilinear direction with locked differential, which allowed the highest traction performance to be achieved. During the tests, engine speed was kept at a constant 1700 rpm (68% rated speed) by means of the hand throttle. This engine speed corresponded to the highest torque and the lowest specific fuel consumption of the engine. Two different gears were used in order to maximise the drawbar power developed: 1S at low slip and drawbar pull, and 4N at high slip and drawbar pull.

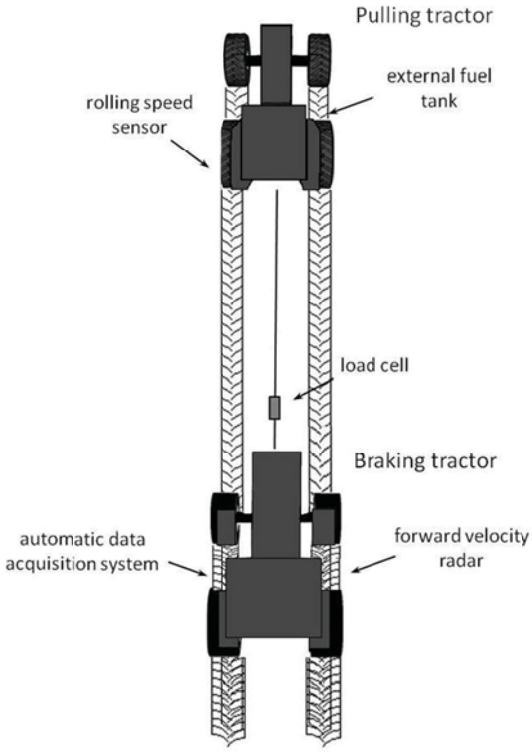

Figure 4. Layout of the traction test

2.4 Characteristics of the Tractors

The specifications of the tractors used in the traction tests are reported in Table 1.

The pulling tractor was a MFWD Hürlimann H488 DT 65 kW weighing 40.8 kN, whilst the braking tractor was a John Deere 6920 weighing 66.7 kN. The pulling tractor was equipped with 380/85R24 front tyres and 420/85R34 rear tyres.

Table 1. Some specifications of the tractors used in the traction performance studies

Braking tractor	John Deere 6920 (110 kW)			
Weight (kN)	66.7			
Pulling tractor	Hürlimann H488 DT (65 kW)			
Weight (kN)	40.8			
Wheelbase L (m)	2.34			
Tyre (front - rear)	380/85R24 - 420/85R34			
Tyre width b (front - rear) (m)	0.38 - 0.44			
Tyre unloaded radius R (front - rear) (m)	0.63 - 0.79			
Rim diameter D_{rim} (front - rear) (m)	0.61 - 0.86			
Tyre carcass stiffness K_{carc} (front - rear) (kN m^{-1})	129.5 - 111.8			
Pressure dependence of tyre ΔK_p (front - rear) (kN m^{-1} kPa^{-1})	1.22 - 2.00			
	L1P1	L1P2	L2P1	L2P2
Height of the drawbar h (m)	0.80	0.83	0.71	0.77
Stationary wheel load W_0 (front - rear) (kN)	9.3 - 11.1	9.3 - 11.1	10.5 - 14.6	10.5 - 14.6
Tyre rolling radius R_r (front - rear) (m)	0.58 - 0.76	0.59 - 0.77	0.58 - 0.76	0.58 - 0.77
Tyre inflation pressure P_{in} (front - rear) (kPa)	60 - 60	160 - 160	60 - 60	160 - 160
Tyre stiffness (front - rear) (kN m^{-1})	203 - 232	325 - 432	203 - 232	325 - 432

Traction performance was measured in four configurations (L1P1, L1P2, L2P1, L2P2) in which tractor weight was varied between 40.8 kN and 50.2 kN with ballasts, and tyre inflation pressure was varied between 60 kPa and 160 kPa (Table 1). The wheel load and tyre pressure values considered are representative for medium powered tractors, and were matched according to the indications of the tyre manufacturer. The load acting on the wheels in the stationary condition was measured with a flat bed wheel load scale (Haenni WL 103, Jegenstorf, Switzerland). Tyre inflation pressure was measured with a Motometer tyre pressure gauge (Mühlacker-Lomersheim, Germany).

The tyre rolling radius R_r (Table 1) was determined according to American Society of Agricultural Engineers (ASAE) Standard S296.2 as the distance travelled per revolution of the wheel divided by 2π when operating at the specified zero condition. The latter was here assumed to be the vehicle operating at zero drawbar pull on a smooth road. This condition allows the travel reduction (slip) to have a fixed base which is not dependent upon the test condition (soil strength) (Wismer & Luth, 1973). Moreover, the difference in measured rolling radii between a hard surface and a test surface is small under normal agricultural soil conditions (untilled soil), and thus has little impact on the final results (Zoz & Grisso, 2003). A significant linear type regression between the rolling radius of the front and rear wheels and the inflation pressure at the two wheel loads considered is shown in Figure 5.

Parameters K_{carc} and ΔK_p (Table 1), which characterise tyre stiffness, were determined on the basis of the tyre specifications as in Lines and Murphy (1991).

The relationship between *PTO* (power-take-off) and fuel consumption in kg/h at 1700 rpm engine speed (Figure 6) was derived in a laboratory test with a torque dynamometer (Schenck W700, Darmstadt, Germany).

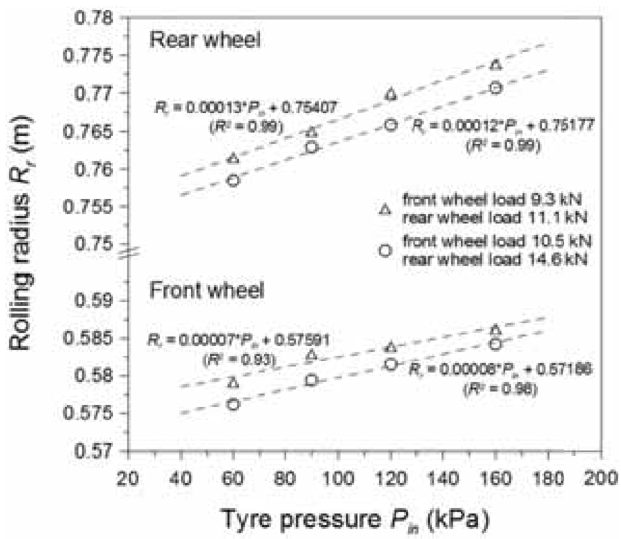

Figure 5. Variation of the rolling radius of the front wheel (380 85R24 tyre) and the rear wheel (420 85R34 tyre) with the inflation pressure at two wheel loads

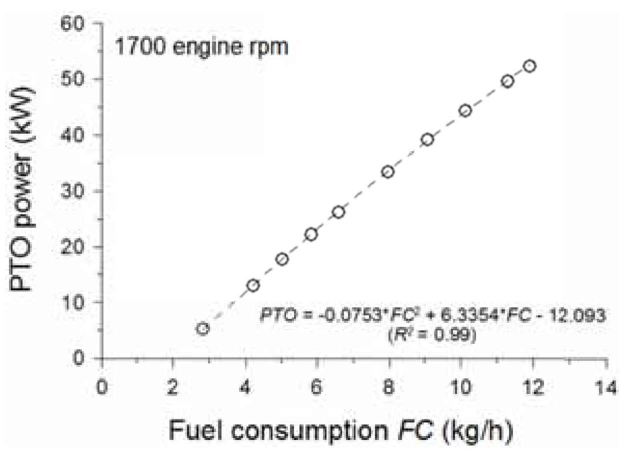

Figure 6. Ratio between PTO and fuel consumption of the Hürlimann H488 DT 65 kW at 1700 rpm engine speed

2.5 Characteristics of the Topsoil

Some physical parameters of the agricultural clay (C) Vertic Cambisol are listed in Table 2, along with the mechanical parameters for the soil-tyre interaction model.

Soil texture was characterised according to the United States Department of Agriculture (USDA) classification system, moreover, soil type was classified according to the Food and Agriculture Organization of the United Nations (FAO) system (2006). Bulk density was measured on undisturbed soil samples according to Blake and Hartge (1986). Volumetric water content was measured via a time domain reflectometry (TDR) device (E.S.I. Environmental sensors MP-917, Sidney, Canada) with two-rod single diode probes.

Topsoil mechanical parameters for the soil-tyre interaction model were derived via vertical plate penetration tests and horizontal plate shear deformation tests with a tractor-mounted bevameter (Bekker, 1960). An exhaustive description of the bevameter used was given by Diserens and Steinmann (2003).

Vertical plate penetration tests were carried out with two circular plates of 20 cm and 30 cm diameter at a penetration rate of around 0.02 m s^{-1}. The cohesive and frictional moduli of deformation K_c and K_φ as well as the exponent of deformation n were determined according to Wong (1980). The horizontal plate shear deformation tests were performed by an annular plate with an outer diameter of 30 cm and an inner diameter of 20 cm at different vertical pressures ranging between 25 and 215 kPa. Measured shear stress-displacement curves were fitted with equation 15, and values of c, φ and k were determined according to the procedure described by Wong (1980).

In order to consider the multipass effect, i.e. the different behaviour of soil interacting with the front and rear wheel, the vertical plate penetration tests and the horizontal plate shear deformation tests were performed before the passage of the tractor, as well as on the rut left by the passage of the front wheel. Because the parameters K_c, K_φ and n calculated before and after the passage of the front wheel changed significantly, they were differentiated for soil interacting with the front wheel ($K_{c,f}$, $K_{\varphi,f}$, n_f) and soil interacting with the rear wheel ($K_{c,r}$, $K_{\varphi,r}$, n_r), as reported in Table 2. A unique characterization was adopted for shear parameters c, φ and k, as they did not change significantly before and after the passage of the front wheel (Table 2).

Table 2. Some characteristics of the clay soil used in the traction performance studies

Soil property	0-0.20 m depth
Sand (g kg^{-1})	200
Silt (g kg^{-1})	320
Clay (g kg^{-1})	480
Texture (USDA classification)	Clay (C)
Soil classification (FAO, 2006)	Vertic Cambisol
Dry bulk density (Mg m^{-3})	1.31
Volumetric water content (%)	27.0
Cohesive modulus of deformation (front wheel) $K_{c,f}$ (kN $m^{-(n+1)}$)	2354.1
Frictional modulus of deformation (front wheel) $K_{\varphi,f}$ (kN $m^{-(n+2)}$)	-4130.0
Exponent of deformation (front wheel) n_f	1.01
Cohesive modulus of deformation (rear wheel) $K_{c,r}$ (kN $m^{-(n+1)}$)	2168.9
Frictional modulus of deformation (rear wheel) $K_{\varphi,r}$ (kN $m^{-(n+2)}$)	-3498.3
Exponent of deformation (rear wheel) n_r	0.79
Cohesion c (kPa)	24.4
Angle of shear resistance φ (°)	18.0
Shear deformation modulus k (m)	0.014

3. Results

3.1 Simulation of Contact Surface and Contact Stresses

The reliability of the soil-tyre interaction model for simulating both wheel sinkage (rut depth) and traction performance was pointed out by Osetinsky and Shmulevich (2004).

The simulation of the soil-tyre contact surface and the contact stresses (normal σ and shear τ) together with the soil strength τ_{max} for the rear wheel at a slip of 10% in the four cases considered, is represented in Figure 7. Soil strength is given by:

$$\tau_{max} = (c + \sigma \tan\varphi) \tag{31}$$

the latter corresponds to the horizontal asymptote of equation 15 and defines the maximum shear stress on soil under a given normal stress.

The geometry of the soil-tyre contact surface is defined by the *x*-axis and the *z*-axis according to the reference system in Figure 1. The stress condition at the soil-tyre contact together with the soil strength is defined by the *x*-axis and the Stress-axis.

The geometry of the contact surface as well as the contact stresses and soil strength varied more noticeably with the increase in tyre pressure than with the increase in wheel load, with the former producing a deeper and shorter contact surface, and the latter creating a deeper and longer one.

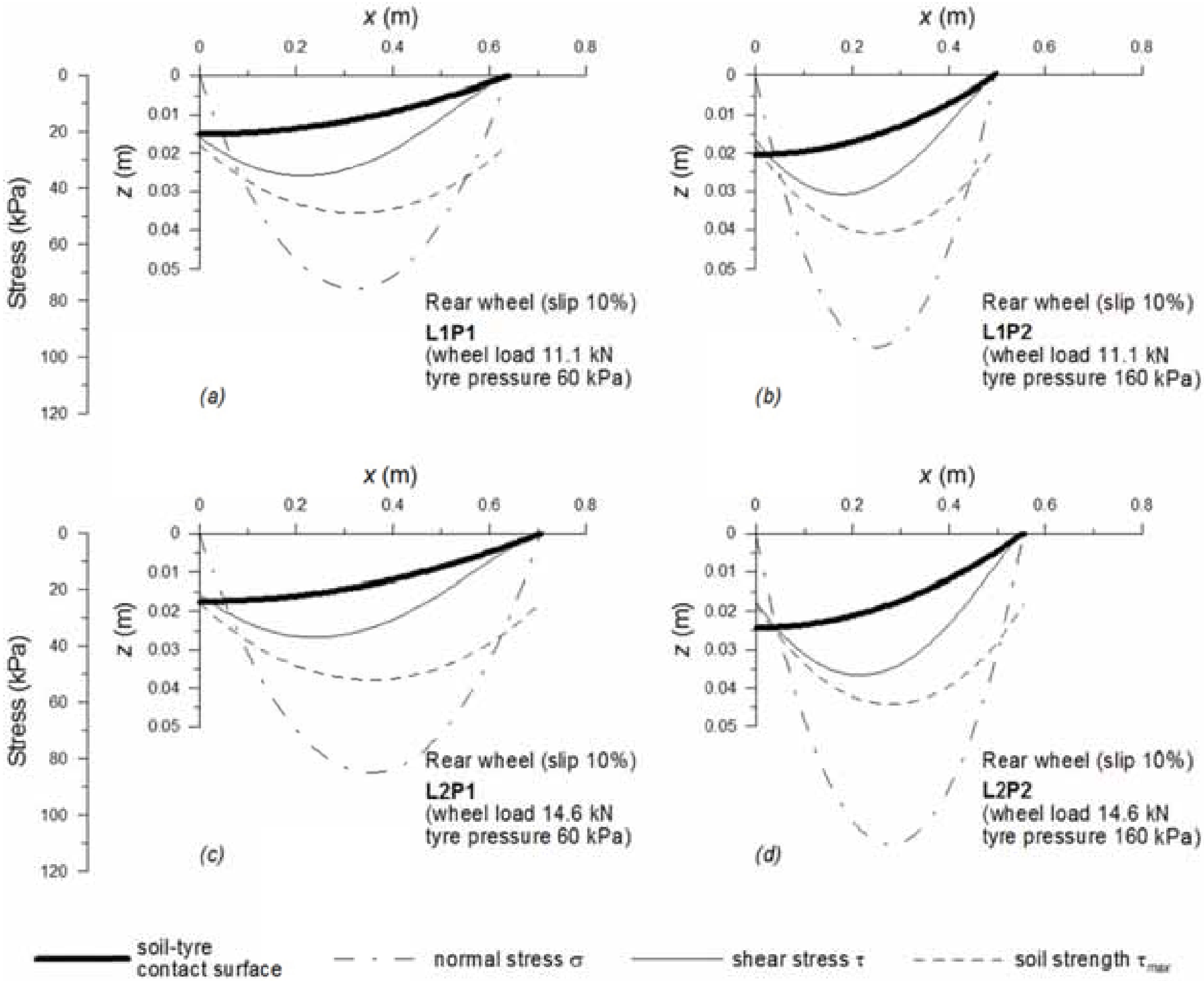

Figure 7. Simulation of the geometry of the soil-tyre contact surface, the contact stresses (normal σ and shear τ), and the soil strength τ_{max} for the rear wheel (420 85R34 tyre) at a slip of 10% in the four configurations considered

3.2 Measurement and Simulation of Drawbar Pull and Traction Coefficient

The measured and simulated traction performance in terms of drawbar pull *DP* and traction coefficient μ_{tr} as a function of slip *i* are reported in Figure 8.

Lowering the inflation pressure produced an improvement in drawbar pull and traction coefficient both with and without ballasting the tractor.

The increase in wheel load resulted in a higher drawbar pull at both 60 kPa and 160 kPa tyre inflation pressure, although it failed to produce noteworthy variations in terms of traction coefficient.

Figure 9 shows the simulation of the traction coefficient as a function of slip (from 4% to 25%) and tyre inflation pressure (from 60 to 160 kPa) both without (Figure 9*a*) and with ballasts (Figure 9*b*). In this simulation, the slip base is assumed to vary according to the variation of the rolling radius reported in Figure 5.

Tyre pressure had a significant effect on traction coefficient, which decreased with increasing pressure. The increase in tractor weight (wheel load) resulted in a slight decrease in traction coefficient.

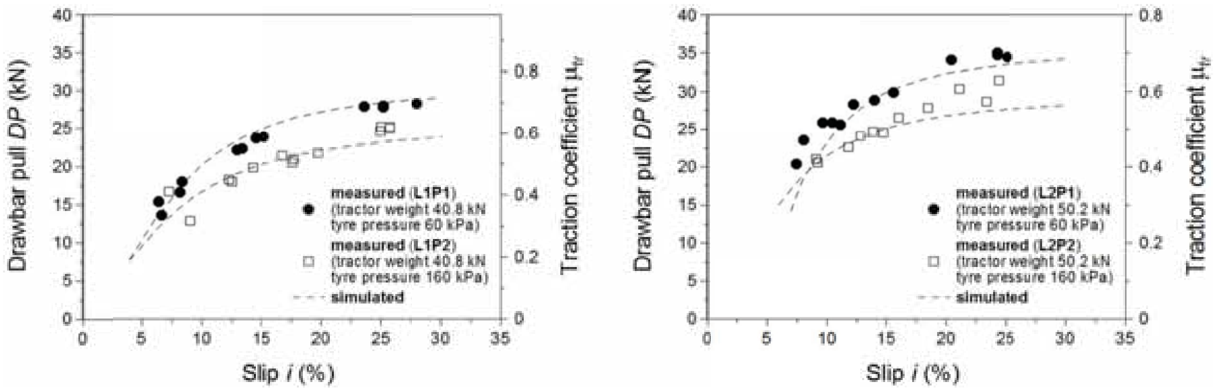

Figure 8. Measured and simulated drawbar pull and traction coefficient as a function of slip in the four configurations considered

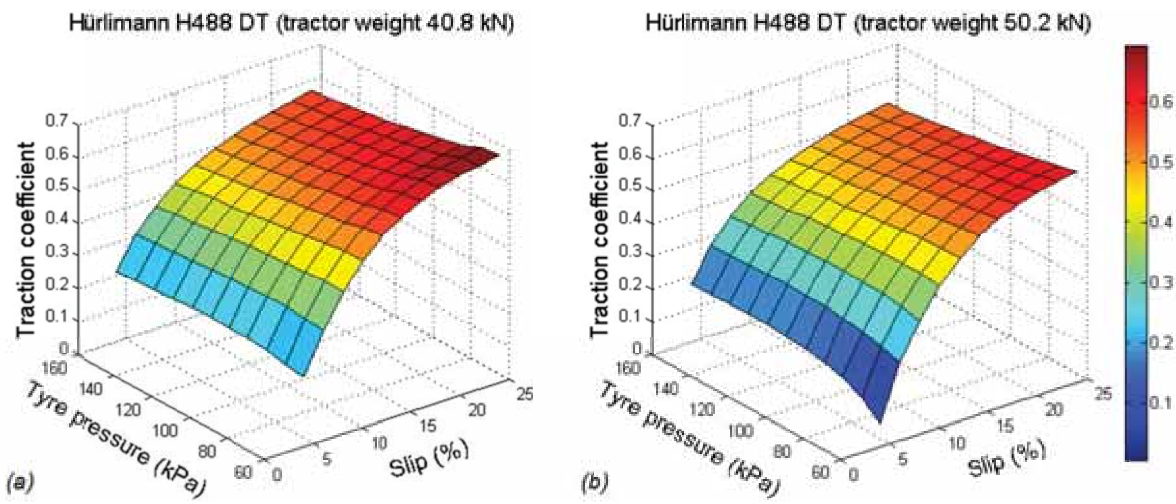

Figure 9. Simulation of the traction coefficient as a function of slip (from 4% to 25%) and tyre inflation pressure (from 60 to 160 kPa) without ballasts *(a)* and with ballasts *(b)*

3.3 Simulation of Tractive Efficiency

In order to properly measure the tractive efficiency η_{tr}, a wheel torque dynamometer - not available for our tests - would be required. Tractive efficiency was therefore merely simulated as a function of slip (from 4% to 25%) and tyre inflation pressure (from 60 to 160 kPa) without ballasts (Figure 10*a*) and with ballasts (Figure 10*b*).

It emerged that tractive efficiency decreased along with an increase in both tyre pressure and wheel load, although the decrease owing to wheel load was less significant.

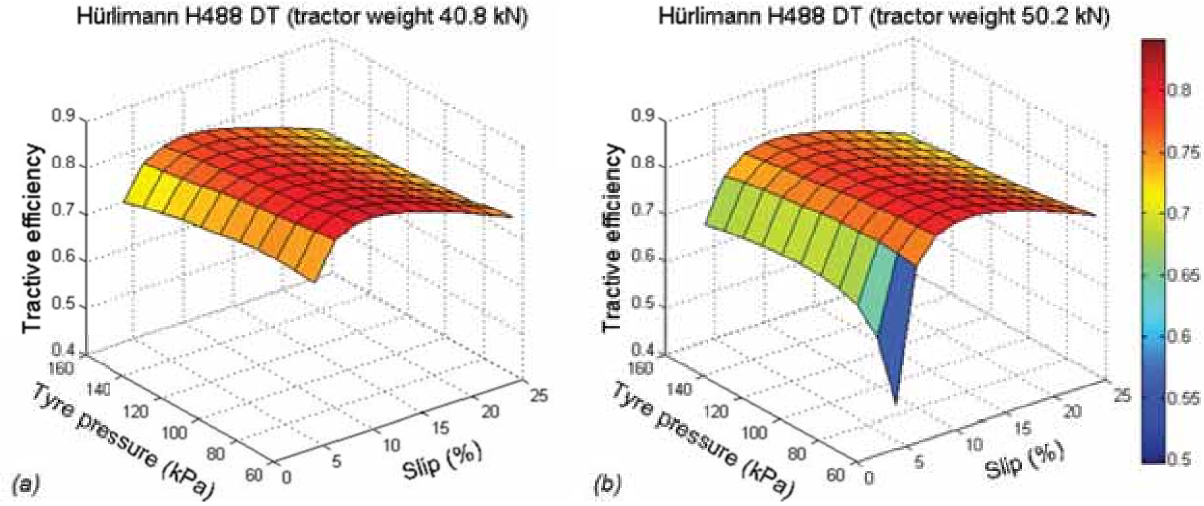

Figure 10. Simulation of tractive efficiency as a function of slip (from 4% to 25%) and tyre inflation pressure (from 60 to 160 kPa) without ballasts *(a)* and with ballasts *(b)*

3.4 Measurement and Fitting of Power Delivery Efficiency and Specific Fuel Consumption

Values for power delivery efficiency η_{PD} and specific fuel consumption *SFC* (drawbar power basis) as a function of the slip are reported in Figure 11 for the four tractor configurations considered (Table 1). The equivalent *PTO* was calculated on the basis of measured fuel consumption, according to Figure 6.

Power delivery efficiency and specific fuel consumption were fitted (least squares method) with the following equations:

$$\eta_{PD} = Ae^{\left(1-\frac{i}{l_A}\right)}\left(\frac{i}{l_A}\right)^{\left(1+\theta_A\frac{i}{l_A}\right)} \quad (32)$$

and

$$SFC = \frac{1}{Be^{\left(1-\frac{i}{l_B}\right)}\left(\frac{i}{l_B}\right)^{\left(1+\theta_B\frac{i}{l_B}\right)}} \quad (33)$$

wherein A, l_A, θ_A and B, l_B, θ_B are fitting parameters which mainly control: the positive peak of η_{PD} (parameter A), the negative peak of *SFC* (parameter B), the slip value at which the peak is reached (parameters l_A and l_B), and the slope of the curve beyond the peak (parameters θ_A and θ_B). Values of these parameters as well as the root mean square error *RMSE* of the fitting are reported in Table 3.

An increase in tyre pressure resulted in lower power delivery efficiency and higher specific fuel consumption at both tractor weights, with a more significant variation at a tyre pressure of 60 kPa.

An increase in tractor weight, at least at a slip of less than 15%, resulted in a notable decrease in power delivery efficiency and an increase in specific fuel consumption at a tyre pressure of 60 kPa, as well as a slight decrease in power delivery efficiency with a more significant decrease in specific fuel consumption at a tyre pressure of 160 kPa.

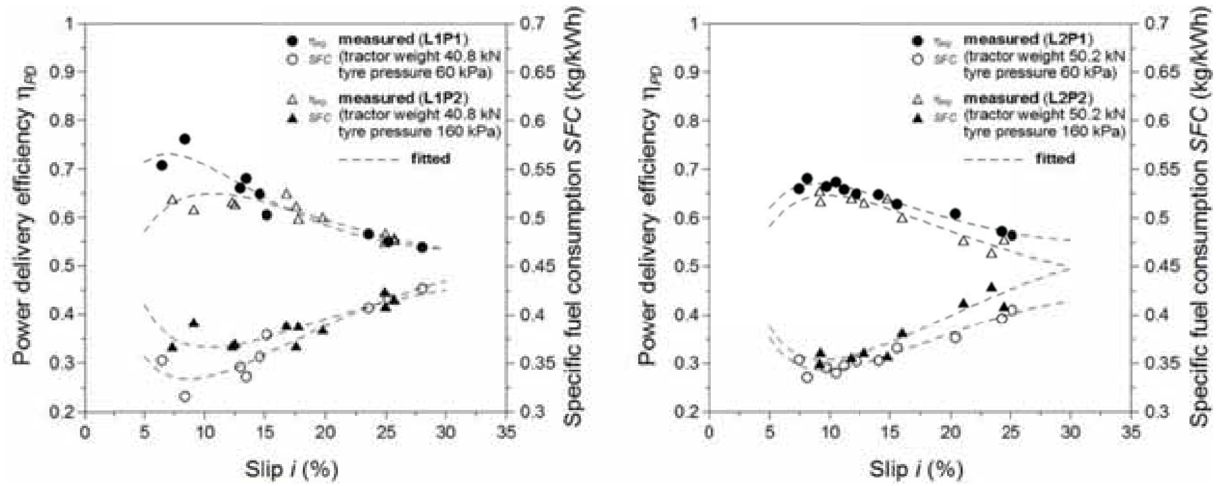

Figure 11. Measured and fitted power delivery efficiency and specific fuel consumption (drawbar power basis) as a function of slip in the four configurations considered

Table 3. Values of parameters A, l_A, θ_A and B, l_B, θ_B, and root mean square error *RMSE* from the fitting of the power delivery efficiency η_{PD} and the specific fuel consumption *SFC*

	η_{PD}				*SFC*			
	A	l_A	θ_A	*RMSE*	B	l_B	θ_B	*RMSE*
	-	-	-	-	(kW h kg^{-1})	-	-	(kg kW^{-1} h^{-1})
L1P1	0.67	3.77	0.28	0.019	2.74	4.62	0.28	0.011
L1P2	0.59	5.98	0.29	0.016	2.47	5.26	0.29	0.010
L2P1	0.61	4.68	0.29	0.007	2.65	4.94	0.29	0.005
L2P2	0.60	5.98	0.27	0.012	2.59	5.08	0.28	0.009

4. Discussion

The advantages in decreasing tyre inflation pressure or ballasting the tractor may be greater or lesser, depending on the change in the interaction between soil and tyre.

It emerged that the traction performance of the tractor considered (Table 1) depended on the geometry of the contact surface between tyre and soil, as well as on contact stresses and soil strength (Figure 7). All of these factors varied significantly with the inflation pressure, and less noticeably with the stationary wheel load in the four configurations considered (Figure 7).

At low inflation pressure (Figures 7*a* and 7*c*), the simulated contact surface was shallow and long. This implied, on the one hand, low soil strength due to low contact pressure, as well as high rolling resistance due to high tyre deformation, and on the other hand, low soil compaction resistance due to low soil sinkage, and the soil strength used on a more extended surface.

According to equation 31, soil strength is given by a cohesive component c and a frictional component $\sigma tan\varphi$ which depends on the normal stress σ. When the inflation pressure was reduced at constant wheel load, the improvement in traction performance was mainly due to the mobilisation of the cohesive component of the soil strength on a more extended contact area, i.e. a higher total contribution of the cohesive component of the soil strength along the contact surface. This allowed better use of the soil strength, which for the same slip resulted in a higher drawbar pull and traction coefficient (Figures 8 and 9), higher simulated tractive efficiency (Figure 10), higher power delivery efficiency, and lower specific fuel consumption (Figure 11).

When the stationary wheel load was increased at a constant tyre pressure, the simulated contact surface varied slightly, becoming longer and deeper (Figures 7*a* and 7*c* and Figures 7*b* and 7*d*). This implied, on the one hand, higher rolling resistance due to greater tyre deformation and higher soil compaction resistance due to greater soil sinkage, and on the other hand, higher soil strength due to greater normal contact stress and the soil strength used on a more extended surface. The improvement of traction performance in terms of drawbar pull (Figure 8) was partly due to a higher total contribution of the frictional component of the soil strength and partly due to a higher total contribution of the cohesive component of the soil strength along the contact surface. In spite of the higher drawbar pull, this way of using the soil strength did not result in any improvement in terms of traction coefficient (Figures 8 and 9), simulated tractive efficiency (Figure 10), or power delivery efficiency (Figure 11). At a slip of under 15%, only the specific fuel consumption decreased with increasing wheel load at a tyre pressure of 160 kPa.

The results of this study may provide helpful indications for an appropriate choice of tractor configuration on cohesive soils in order to optimise tractor performance, thereby saving time and reducing the costs of tillage management.

5. Conclusions

The present study aimed to analyse the effects of variations in tyre inflation pressure and wheel load on the traction performance of a 65 kW MFWD tractor on an agricultural clay (C) Vertic Cambisol.

In the conditions examined, although the tractor developed higher drawbar pull both when tyre inflation pressure was decreased and wheel load was increased, only the decrease in tyre pressure produced improvements in terms of coefficient of traction, tractive efficiency, power delivery efficiency, and specific fuel consumption, while the only significant benefit due to the increase in wheel load was a reduction in the specific fuel consumption at a tyre pressure of 160 kPa and a slip of under 15%. A mechanistic interpretation of these results was proposed.

Two equations describing the relationships power delivery efficiency-wheel slip and specific fuel consumption-wheel slip were presented.

The results of this study may provide helpful indications for an appropriate choice of tractor configuration, as well as for the reasonable control of wheel slip, with a view to optimising traction performance on cohesive soils, thereby saving time and reducing the costs of tillage management.

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Crop Load and Time of Thinning Interact to Affect Fruit Quality in Sweet Cherry

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Abstract

Balanced crop load is key to the production of export-quality cherries. We investigated the level and timing of crop load regulation on fruit quality. Additionally we sought to investigate possible correlation between firmness estimated by compression test or flesh penetrability. Fruit diameter was similar between 1 and 2 bud/spur treatments but was significantly lower in the 4 bud/spur treatment at all thinning times in 'Van' in the 2010/11 season. In contrast 'Sweetheart' fruit diameter was only decreased at 6 and 8 WAFB in the 4 bud/spur treatment in 2010/11. This decrease in 'Sweetheart' was associated with significantly higher soluble solids and starch reserves in leaves, stem, trunk and roots 2-weeks post-harvest in trees thinned at dormancy, relative to trees thinned 8 WAFB. Fruit flesh firmness significantly increased with decreased crop load irrespective of time of thinning in 'Van' in 2010/11. In contrast flesh firmness was significantly higher in the 1 bud/spur treatment and similar between other treatments in 'Sweetheart' in 2010/11. In 2011/12 flesh firmness, soluble solids and colour significantly increased whilst fruit weight and TA significantly decreased 28 days post-harvest relative to at-harvest values. We found strong correlation between values obtained with the FirmTech II and the Guss fruit texture analyser. Sweet cherry fruit quality is optimised through attaining crop load of approximately 10 fruit per cm^2 of limb cross-sectional area through thinning at dormancy or full bloom.

Keywords: carbohydrate, cherry, crop load regulation, post-harvest, thinning

1. Introduction

Sweet cherry is a high value crop where quality can attract significant premiums. High grower returns are particularly important for economic sustainability in countries with high production costs. Due to premium returns in export markets, sweet cherry fruit from Australia are shipped overseas. To maximize returns, and ensure the retention of fruit quality, in overseas markets, growers must deliver large, firm fruit with high sugar levels. Horticulturally, crop load regulation is a key to producing fruit with desired quality attributes (Link 2000) as over-cropped trees produce small, soft fruit due to limited carbohydrate and nutrient supply (Proebsting & Mills, 1981; Whiting & Lang, 2004).

Regulation of crop load can be achieved by several methods: hand-thinning, chemical thinning, mechanical thinning, artificial spur extinction (removal of complete spur from the branch; Ayala & Andrade, 2009) or bud thinning. Hand-thinning is expensive and time consuming (Childers, 1983). Chemical blossom thinning is becoming more widespread, however there are limited options available and many Australian growers are wary of thinning during the bloom period because of the potential risk of over-thinning from subsequent frost damage. The caustic blossom thinner ammonium thiosulfate (ATS) is gaining in popularity, but timing of application is critical and can be difficult in practice in addition to uncertainty regarding the efficacy of thinning (Bound & Jones, 2004; Whiting et al., 2006). There is conflicting evidence on the impact of spur extinction and bud thinning on fruit quality and yield. Whiting and Lang (2004) reported a reduction in yield and increase in fruit size, firmness and sugar content in the 'Bing' variety on dwarfing Gisela 5 rootstock trained to a free-standing, multiple leader open-centre system, however Ayala and Andrade (2009) saw no response to spur thinning in 'Lapins' on the vigorous Mazzard F-12/1 rootstock trained to a central leader system. These authors speculated that the lack of response in Lapins/F-12/1 trees was due to fruit sink limitation or competition by vegetative sink. Einhorn et al. (2011) similarly reported no consistent quality responses to varying crop load on variety ‘Sweetheart’ on Mazzard F-12/1 and Measham et al. (2012) did not see any positive effect on fruit size or sugar accumulation after thinning in ‘Regina’ on Mazzard F-12/1 trained to a bush system. In a study of ‘Lapins’ on Maxima 14 rootstock, a positive effect on fruit size distribution following spur and bud thinning was observed, but without a reduction in yield (von Bennewitz et al., 2010), whereas yields were reduced on Sweetheart/F-12/1 after bud thinning such that the lowest crop loads returned the lowest returns to the grower in two consecutive seasons (Einhorn, 2011). Neilsen et al. (2007) make the observation that spur extinction results in permanent yield reductions, regardless of factors that may alter annual fruit set and additionally, that fruit firmness was only improved in lower cropping trees in one season. Measham et al. (2012) found no interaction or main effect of crop load intensity or timing of thinning on fruit firmness, but an interaction was evident on fruit size. The potential for crop load manipulation to positively influence fruit quality seems to be highly dependent on variety, rootstock and training system, as well as the level of crop load set.

A recently developed training system, known as the Kym-Green-Bush or KGB (Green, 2005), is comprised of 10-15 branches arising from the main trunk which is headed in the first year at approximately 0.3 m from soil level. This system spreads vigour, provides superior internal rate of return compared to trellised Tatura or Sol-Axe systems (Close et al., in press), and relies on vegetative shoots only on the branches in addition to spur leaves for carbohydrate supply.

The objective of this study was to investigate the impacts of both crop density, and timing of crop load regulation by bud, flower or fruitlet thinning, on fruit quality of ‘Van’ and ‘Sweetheart’ on Mazzard F-12/1 rootstock trained to the KGB system. It was hypothesized that, given the lack of lateral shoots and therefore potentially low carbohydrate supply, fruit quality would be improved by thinning, and that crop load effects on fruit quality would be less apparent with later thinning due to lost carbohydrate resource invested in bloom and fruitlets. Additionally we sought to investigate possible correlation between firmness estimated by the FirmTech II or the GÜSS Fruit Texture Analyser.

2. Methods

Three trials were conducted on commercial orchards in southern Tasmania, Australia; at Plenty in the Derwent Valley (42°71'S, 146°90'E) , and Old Beach (42°71'S, 146°90'E) over two consecutive seasons on mature, regular bearing ‘Van’ and ‘Sweetheart’ sweet cherry trees. Studies commenced in spring of 2010.

In the 2010/11 season, Trial 1 was conducted on 18-year-old ‘Van’ trees at Plenty and Trial 2 on nine-year-old ‘Sweetheart’ trees at Old Beach, near Hobart. The following season (2011/12) Trial 3 was conducted on nine-year-old ‘Van’ trees at Plenty.

These varieties were chosen to represent a commonly grown variety (Sweetheart) and a variety that generally has a higher crop load (Van) than other varieties.

All trees were on F-12/1 rootstocks and pruned to a KGB system. Row orientation in all trials was north-south, with a planting spacing of 4.8 m x 2.5 m in Plenty, and 4.3 x 2.0 m in Old Beach. Trees were subjected to standard orchard management with respect to irrigation, fertilisation and pest management.

2.1 Experimental Design

For each trial, trees were selected while dormant. Trees were blocked into groups depending on position within the row, and treatments were allocated at random to single tree plots within each block in a randomised complete block design. There were six replicates in Trials 1 and 2 and five replicates in Trial 3. In Trials 1 and 2 whole trees were used as the experimental unit whereas in Trial 3, two representative limbs were chosen on opposite sides of each tree.

2.2 Treatments

In all trials, buds/flowers or fruitlets were removed by hand (randomly selected given that we have previously tagged buds at dormancy and shown no effect on subsequent fruit quality) from whole trees to each crop load level at each thinning time as follows;

Trial 1: Crop load levels were achieved by thinning to 1, 2 or 4 buds per spur at pre-bloom (approximately 28 days prior to full bloom), full-bloom (FB), 2 weeks after FB (WAFB), 4 WAFB, or 6 WAFB, giving a factorial arrangement of three crop loads x five thinning times.

Trial 2: Crop load treatments were applied as in Trial 1 with an additional thinning time of 8 WAFB, giving a factorial arrangement of three crop loads x six thinning times.

Trial 3: Crop load levels were achieved by thinning to 2 or 4 buds per spur or left with the natural crop load at pre-bloom or 6 WAFB, giving a factorial arrangement of three crop loads x two thinning times.

2.3 Sampling

Harvest of experimental plots occurred in the mornings and was coordinated with commercial harvest in each orchard. All fruit from Trial 1 was picked into lugs by the grower (any rotten fruit was discarded by the pickers). Fruit for each tree was weighed in the field using a Salter Model 235 6s hanging scale suspended from a tripod and a subsample of at least 300 fruit taken for further analysis. Due to adverse weather conditions prior to harvest, Trial 2 was abandoned by the grower. The harvest regime was modified such that 2 limbs were harvested on each tree and total number and weight of fruit recorded separately for each limb. In Trial 3, all fruit was harvested from two marked limbs. Trunk (TCSA) and limb (LCSA) cross-sectional areas were calculated from circumference; measured at 4 cm above the graft union or at the base for trunk and limb respectively. Estimation of total yields in each trial was based either on total weight but not total fruit number (Trial 1) or as a multiple of fruit numbers per branch.

Harvested fruit from each trial was returned to the laboratory, weighed and sorted as either marketable, cracked or otherwise. From this data percent fruit cracked was calculated. For quality assessments a subsample of 25 blemish-free fruit was taken from each replicate of Trials 1 and 2, and 30 fruit from Trial 3.

Leaf (fully expanded on outer canopy) and wood samples (one sample from each aspect = 4 samples per tree) for analysis of TSS and starch were taken two weeks after harvest from all trial trees thinned at dormancy and 8 WAFB in Trial 2 only. Two branches (east and west aspect) and the main trunk were sampled with a tree corer, whilst woody root material was sampled using a soil auger.

2.4 Assessments (Quality)

Fruit diameter was measured using digital vernier calipers and fruit weight was recorded using an AND digital balance (model GX-4000). Skin colour was assessed using the CTIFL (Centre Technique Interprofessionnel des Fruits et Légumes) color chart for cherries. A GÜSS Fruit Texture Analyser, model GS-20, fitted with a 2 mm penetrometer probe, operating at a penetration speed of 10mm/second and a penetration depth of 4mm, was used to measure flesh firmness (on pared flesh) and skin puncture force. In the second year, fruit firmness was also assessed using a Bio Works FirmTech II Fruit Firmness Tester, and stem retention was assessed by determining the stem pull force measured with a stand mounted Mark 10 force gauge. The incorporation of the FirmTech II into our fruit quality assessment allowed for investigation of correlation between values obtained with this and the GÜSS Fruit Texture Analyser.

Fruit from each replicate were then juiced collectively and 3 individual samples taken for measurement of total soluble solids (TSS), pH and titratable acidity (TA). TSS concentration (^{o}Brix) was assessed with an Atago PR-1 digital refractometer, and juice pH and TA were determined using a Mettler Toledo G20 compact titrator.

In Trial 3, a further subsample of 30 fruit was taken from each replicate, placed into polyethylene bags which were then sealed to maintain the ambient environment at close to 100% relative humidity and then stored at 0°C with no atmospheric control. These fruit were assessed for post-harvest quality attributes as above after 28 days storage.

2.5 Assessments (Carbohydrate Analysis)

Soluble sugars were extracted from 100 mg of dried, powdered leaf or wood tissue. For each sample, 3 mL of 80% (v/v) ethanol was added and incubated at 60°C. The samples were then centrifuged at 4000 g for 10 min at 8°C. The supernatant liquid was kept in a separate tube and the pellets were again extracted similarly, twice more. The supernatants were combined and frozen until analysis for soluble fructose, glucose, sucrose, and sorbitol using HPLC – MS (Mehouachi et al., 1995). The residue was then analysed for starch (*St*) concentration. Starch remaining in the undissolved pellet of plant material after ethanol extractions were enzymatically (amyloglucosidase; Fluka-10115, Sigma-Aldrich, St Louis, MO, USA) reduced to glucose using the method detailed by Palacio et al. (2007). The concentration of starch was determined by a phenol-sulfuric acid colorimetric assay (Dubois et al., 1956) as modified by Buysse and Merckx (1993).

2.6 Data Analysis

Data were subjected to Analysis of Variance and/or Linear Regression analysis using Genstat 12.1 (VSN International Ltd). Data are presented as mean values for each treatment. Significance was calculated at P = 0.05

and least significant difference (LSD) used for comparison of mean values in the tables and figures. No data transformations were necessary.

3. Results

3.1 Crop Yields

There were significant differences in estimated yield efficiency (Table 1) with level of bud thinning in Van in 2010/11 ($F_{2,74}$=46.14, p<0.001) and 2011/12 ($F_{2,58}$=5.42, p=0.007) but timing of thinning was not significant and there were no significant interactions. Reducing bud density showed similar trends in both cultivars in the first season (Trials 1 and 2), with the 1 bud/spur treatment resulting in approximately half the crop load (represented as number of fruit per cm^2 LCSA) of the higher thinning level (Figure 1). Natural fruit set in the second season (Trial 3) was very low; thinning to 2 buds/spur in this trial reduced crop load to approximately 50% of the natural set (Figure 1, Table 1).

Table 1. The effect of bud thinning on yield efficiency (g fruit per cm2 tree cross-sectional area (TCSA) for Trial 1 and limb cross-sectional area (LCSA) for Trials 2 and 3) in 'Van' and 'Sweetheart'. Bars represent one standard error of the mean. Note crop load in the Van 2010/11 trial is based on TCSA of 'Van' and 'Sweetheart' cherry varieties on F-12/1 rootstocks and pruned to a KGB system. Means within columns followed by different letters are significantly different as calculated by least significant difference at P = 0.05 using Genstat 12.1 (VSN International Ltd)

	Yield efficiency (g/cm^2)		
	Trial 1: Van (2010/11; TCSA)	Trial 2: Sweetheart (2010/11; LCSA)	Trial 3: Van (2011/12; LCSA)
1 bud/spur	109 a	114 a	-
2 buds/spur	149 b	170 b	79 a
4 buds/spur	222 c	215 c	108 ab
natural	-	-	123 b

As shown in Table 1, yield efficiency (g fruit/cm^2) in 'Sweetheart' and 'Van' sweet cherry.

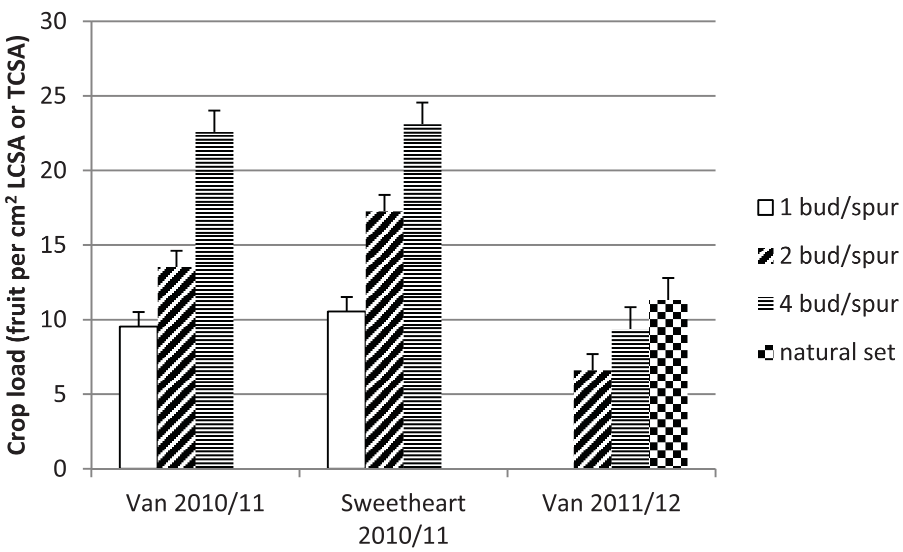

Figure 1. The effect of bud thinning on crop load (number of fruit per cm^2 limb cross-sectional area (LCSA)) in 'Van' and 'Sweetheart'. Bars represent one standard error of the mean. Note crop load in the Van 2010/11 trial is based on TCSA

3.2 Quality at Harvest

Fruit diameter, flesh firmness, skin puncture force, TSS, and TA negatively correlated with fruit/cm^2 LCSA in Trials 2 and 3 (Table 2). Similarly, FirmTech and stem pull values negatively correlated with fruit/cm^2 LCSA in Trial 3 (Table 2).

Table 2. Correlations of quality attributes with crop load (number of fruit per cm^2 tree cross-sectional area (TCSA) for Trial 1 and limb cross-sectional area (LCSA) for Trials 2 and 3) of 'Van' and 'Sweetheart' cherry varieties on F-12/1 rootstocks and pruned to a KGB system

	'Van' 2010/11	r^2	p	'Sweetheart' 2010/11	r^2	p	'Van' 2011/12	r^2	p
Fruit diameter (mm)	=-0.2139x+31.754	0.83	0.0001	=-0.0732x+29.104	0.38	0.0067	=-0.1029x+25.964	0.57	0.0839
Flesh firmness (kg/cm^2)	=-0.0017x+0.1231	0.76	0.0001	=-0.0027x+0.1647	0.68	0.0001	=-0.0012x+0.1061	0.65	0.0517
Skin puncture force (kg/cm^2)	=-0.0049x+0.426	0.68	0.0001	=-0.007x+0.4698	0.72	0.0001	=-0.0034x+0.4121	0.76	0.0236
Firmtech (g/mm^2)	-			-			=-2.2772x+337.8	0.84	0.0099
TSS (°Brix)	=-0.1642x+10.109	0.79	0.0001	=-0.2114x+21.335	0.58	0.0003	=-0.0694x+19.113	0.15	0.4551
TA (g/100g)	=-0.1118x+11.219	0.75	0.0001	=-0.0338x+9.2103	0.16	0.1052	=-0.0425x+12.79	0.25	0.3117
Stem pull force (N)	-			-			=-12.713x+717.76	0.60	0.0700

As shown in Table 2, correlations of quality attributes with fruit load per trunk (Trial 1) and limb (Trials 2 and 3) cross-sectional area (cm^2) (x).

There was a significant interaction effect of thinning level and timing of thinning ($F_{8,2249}$=9.17, p<0.0001) on fruit diameter in Trial 1; 'Van' in 2010/11 (Figure 2). However fruit diameter from trees which were thinned to the 1 and 2 buds/spur treatments generally followed similar patterns, whilst diameter was generally lower from the 4 buds/spur treatment at all thinning times. Similarly, there was a significant interaction effect of thinning level and timing of thinning ($F_{10,2249}$=12.83, p<0.0001) on fruit diameter in Trial 2; 'Sweetheart' in 2010/11 (Figure 2). However in contrast to 'Van', fruit diameter was generally only lowered by the 6 and 8 WAFB in the 4 bud/spur treatment. Different responses to time of thinning between varieties were observed. A general decrease in size was observed in fruit from trees thinned later than FB in 'Van', irrespective of crop load, but no such general decrease was observed in 'Sweetheart'. In Trial 3, thinning time had no effect on fruit diameter (results not presented), but level of crop load had a significant effect ($F_{2,892}$=20.53, p<0.0001) with larger fruit occurring at the lowest crop load. Fruit size in general was higher in 'Van' in 2011/12 than in 2010/11. Mean fruit weight was inversely related to crop load in Trial 3 (weight = 13.13 – 0.056 * crop load; R^2 = 0.79). Fruit weight was also positively correlated with fruit diameter (weight = 0.82 x diameter – 12.30, r^2 = 0.92). There were no effects of crop load or timing of thinning on fruit colour (results not shown).

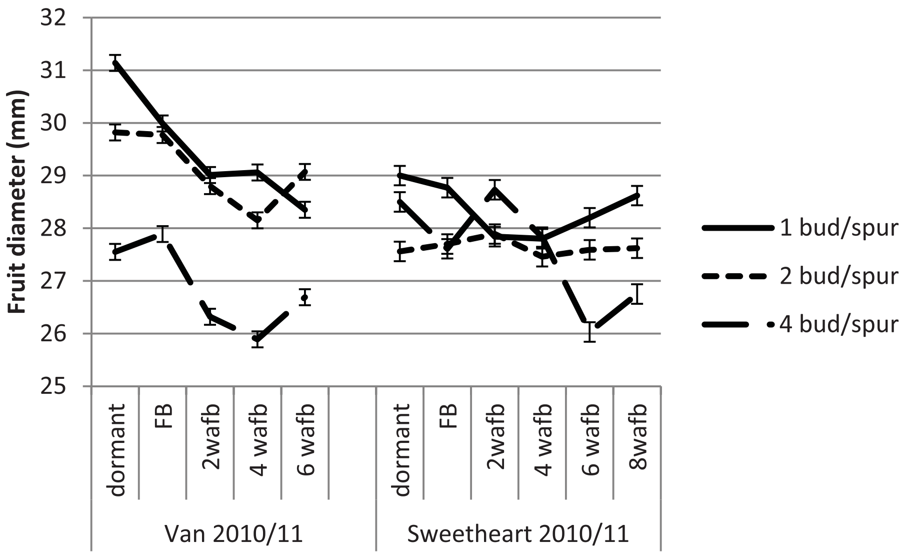

Figure 2. The effect of time and level of bud thinning on fruit diameter of 'Van' and 'Sweetheart' cherry (Trials 1 and 2 – 2010/11). Bars represent one standard error of the mean

There was a significant interaction of crop load and timing of thinning on fruit flesh firmness in Trial 1 ('Van' in 2010/11; $F_{2,2249}$=6.66, p<0.001). Fruit flesh firmness generally increased as crop load decreased but this trend was far less marked in Trial 3 ('Van' in 2011/12; crop load treatment; $F_{2,899}$=6.91, p<0.001) (Figure 3a). There was a significant interaction of crop load and timing of thinning on fruit flesh firmness in Trial 2 ('Sweetheart' in 2010/11; $F_{10,2249}$=10.31, p<0.001), although in contrast to 'Van' in 2010/11 fruit flesh firmness was considerably elevated in the 1 bud/spur treatment compared with other crop load treatments (Figure 3b).

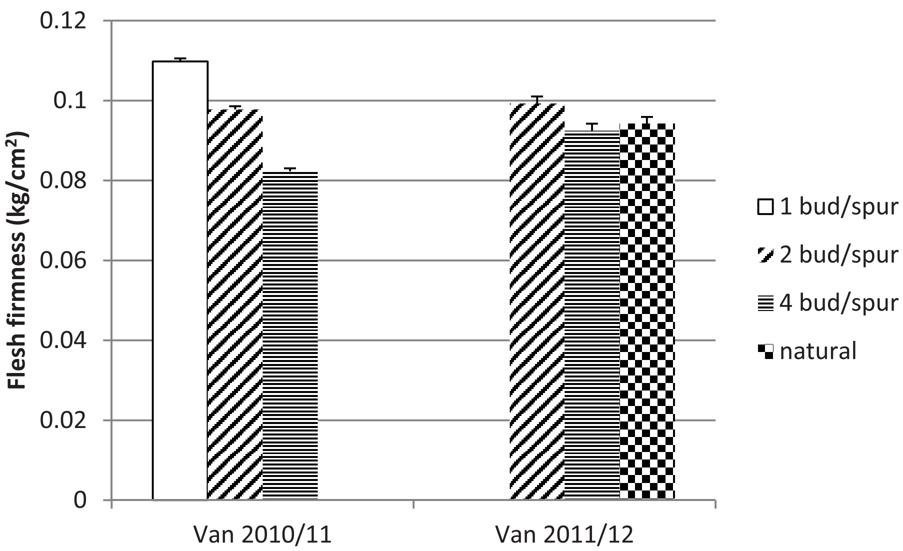

Figure 3a. The effect of bud thinning on fruit firmness of 'Van' cherry (Trials 1 and 3). Bars represent one standard error of the mean

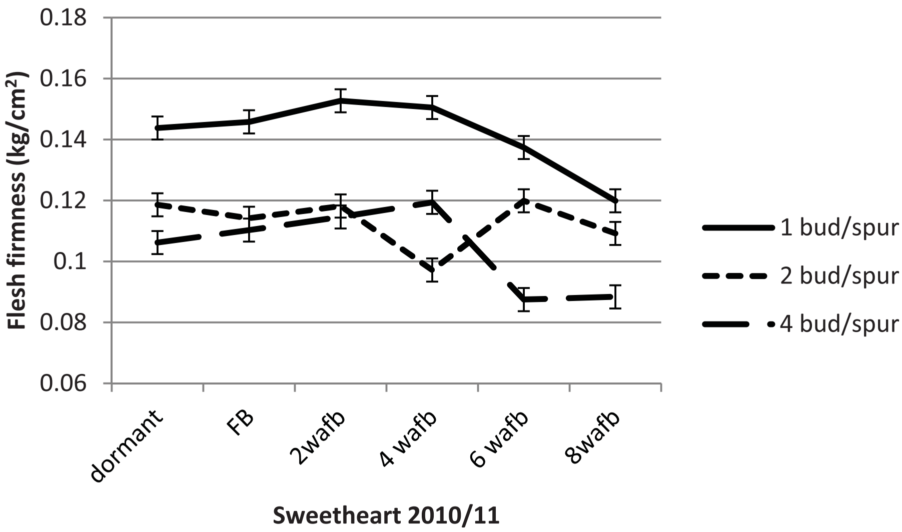

Figure 3b. The effect of time and level of bud thinning on fruit flesh firmness of 'Sweetheart' cherry (Trial 2). Bars represent one standard error of the mean

There were significant interaction effects for crop load and timing of thinning on soluble solids and TA in Trials 1 ($F_{8,269}$=4.13, p=<0.001 and $F_{8,269}$=4.69, p=<0.001, respectively) and 2 ($F_{10,269}$=6.13, p=<0.001 and $F_{10,269}$=4.66, p=<0.001, respectively). The results showed similar general trends as flesh firmness in both varieties (Figures 4a, b, 5a, b). In Trial 3 we found no interaction and a significant effect of crop load only on soluble solids ($F_{2,59}$=3.48, p=0.039) and no effects on TA.

There was significant effect of crop load (but no effect of timing of thinning) on percent fruit cracking in Van in 2010/11 ($F_{2,74}$=51.20, p=<0.001; Figure 6) but not in 2011/12. There was a significant effect of crop load on cracking in Sweetheart in 2010/11 also, but this was part of another study that has been published elsewhere (Measham et al.,2012).

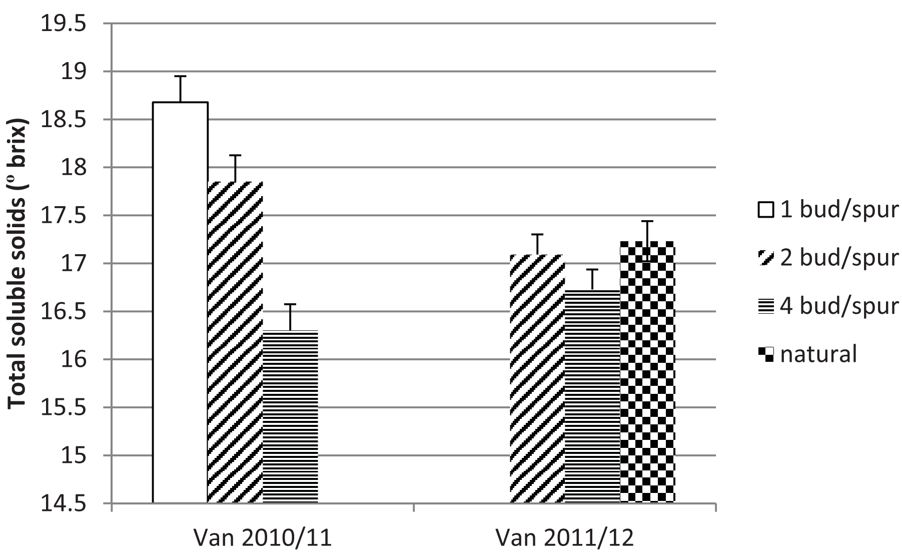

Figure 4a. the effect of bud thinning on sugar content of 'Van' cherry fruit (Trials 1 and 3). Bars represent one standard error of the mean

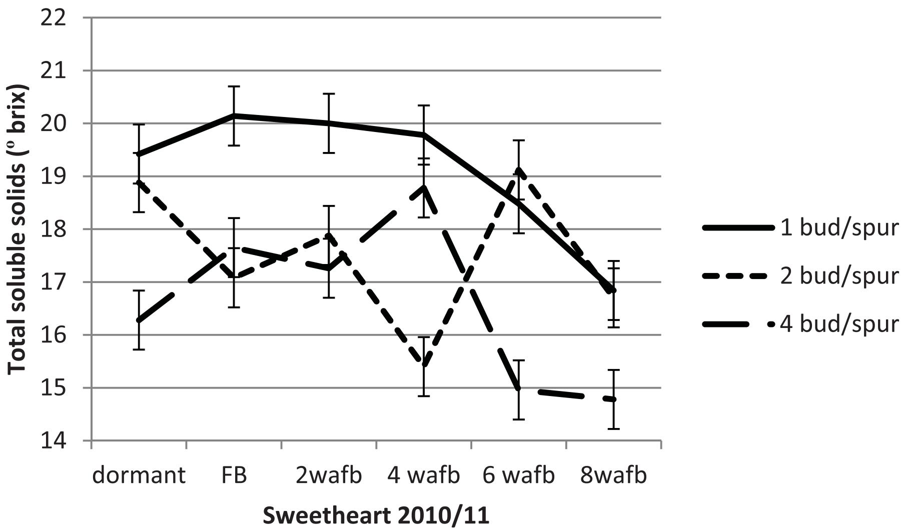

Figure 4b. the effect of time and level of bud thinning on sugar content of 'Sweetheart' cherry fruit (Trial 2). Bars represent one standard error of the mean

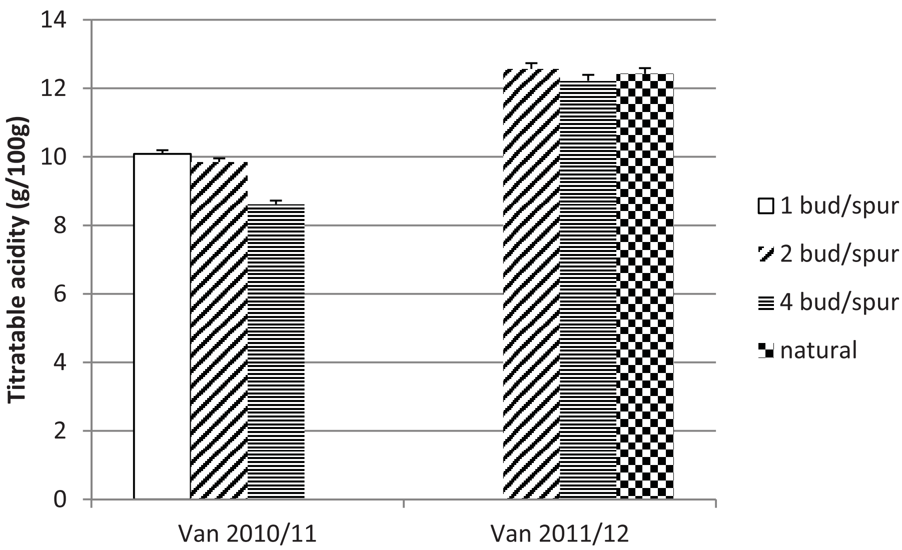

Figure 5a. the effect of bud thinning on titratable acidity of 'Van'cherry fruit (Trials 1 and 3). Bars represent one standard error of the mean

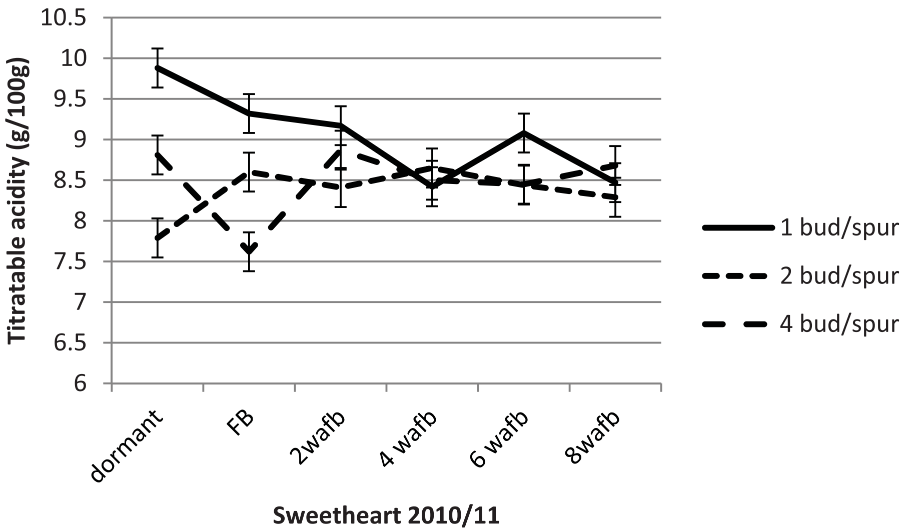

Figure 5b. the effect of time and level of bud thinning on titratable acidity of 'Sweetheart' cherry fruit (Trial 2). Bars represent one standard error of the mean

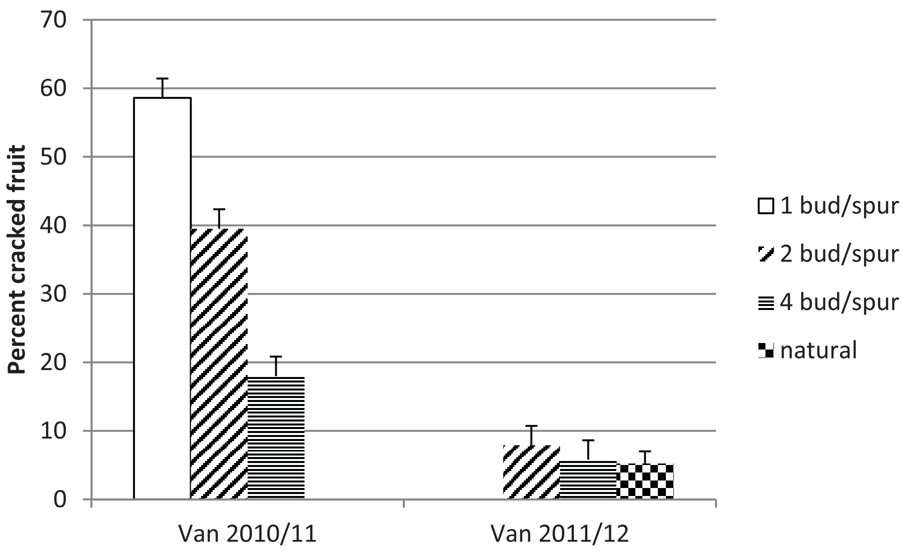

Figure 6. The effect of bud thinning on percent cracked fruit of 'Van' cherry fruit (Trials 1 and 3). Bars represent one standard error of the mean

3.3 Quality Post-Harvest

Fruit had significantly higher flesh firmness and skin puncture values 28 days post-harvest relative to fruit at harvest (Figure 7). Weight and TA significantly decreased whilst colour and TSS significantly increased during storage (Figure 8). Flesh firmness (flesh firmness = 0.0004 * firmtech – 0.04, r^2=0.92) and skin puncture (skin puncture = 0.0011 * firmtech + 0.03, r^2=0.93) correlated with FirmTech values, irrespective of assessment at harvest or post-harvest.

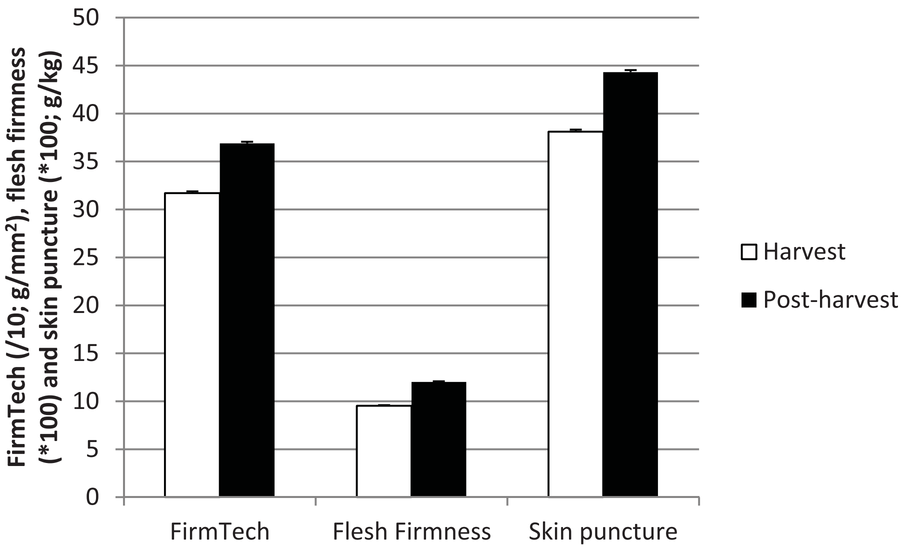

Figure 7. Differences in fruit firmness (FirmTech), flesh firmness and skin puncture force in 'Van' fruit at harvest and 28 days post-harvest (Trial 3). Bars represent one standard error of the mean

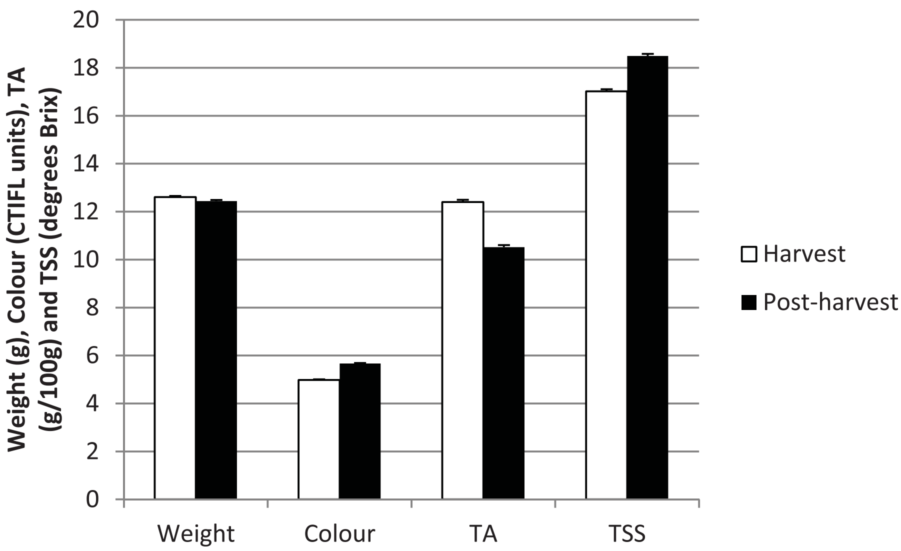

Figure 8. Differences in fruit weight, skin colour, titratable acidity (TA) and total soluble solids (TSS) in 'Van' fruit at harvest and 28 days post-harvest (Trial 3). Bars represent one standard error of the mean

3.4 Crop Load and Carbohydrates

'Sweetheart' trees thinned at dormancy contained significantly greater soluble solids and starch at two weeks postharvest in leaves, stem, trunk and roots than trees thinned 8 WAFB (Figure 9). Trees thinned while dormant had double the TSS in leaves compared to roots, while branch and stem levels were approximately half that of roots. A different pattern was seen in the late-thinned trees, with leaf and roots having similar sugar levels.

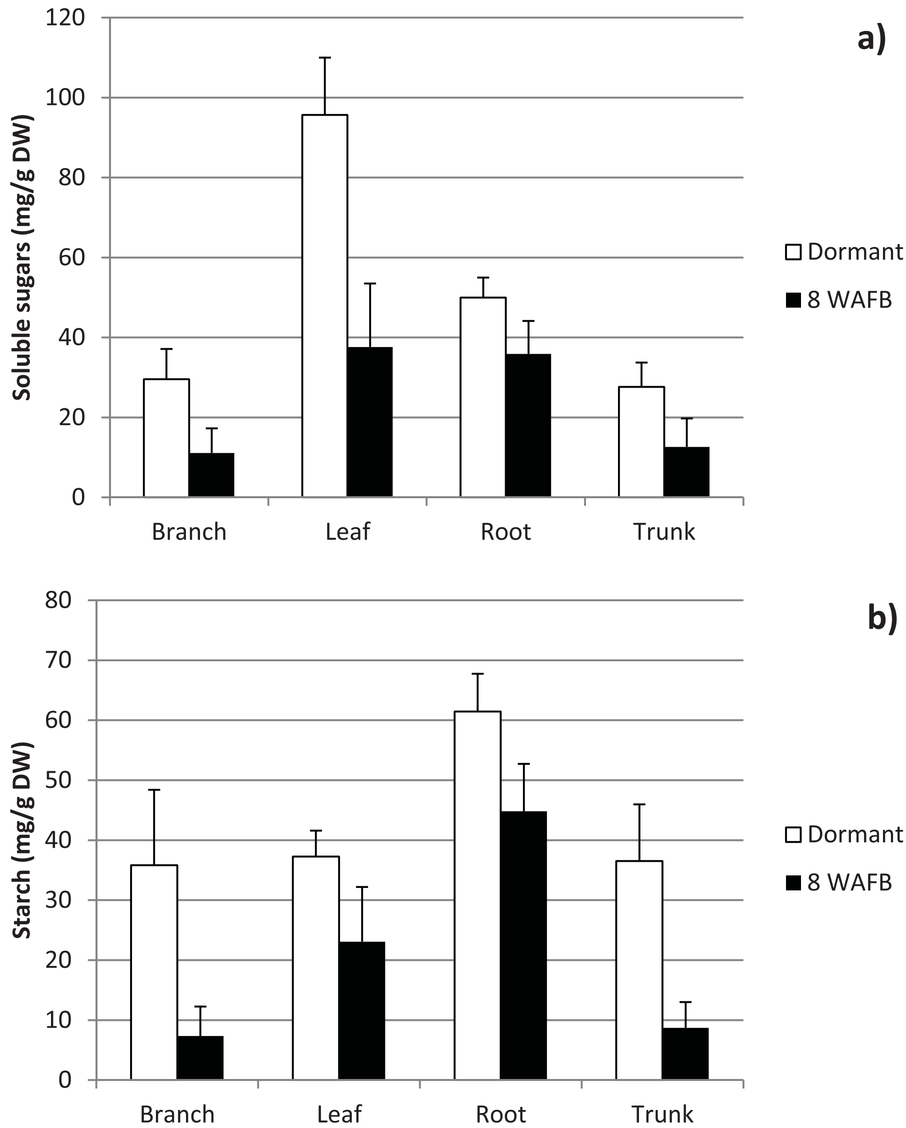

Figure 9. Effect of thinning buds at dormancy (Dormant) and 8 weeks after full bloom (8 WAFB) on 'Sweetheart' tree branch, leaf, root and trunk TSS (a) and starch (b) levels sampled 14 days post-harvest (Trial 2). Bars represent one standard error of the mean

3.5 Correlation of Firmness Values from GÜSS Fruit Texture Analyser and Firmtech II

A correlation of fruit flesh puncture values with fruit compression test values yielded a liner relationship (Figure 10; y=0.0004x-0.0416, R^2=0.92442, p<0.0001).

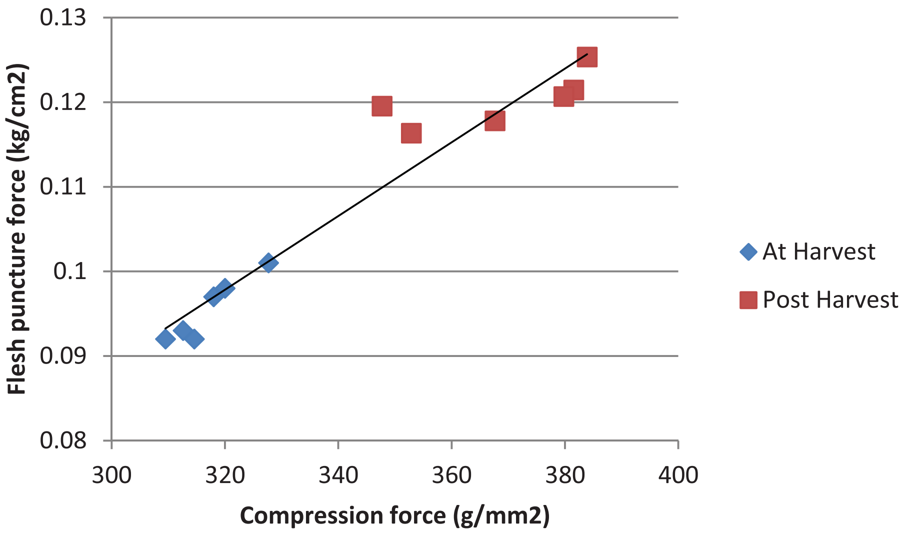

Figure 10. Flesh puncture force (measured with the GÜSS Fruit Texture Analyser) correlated against whole fruit compression force (measured with the Firmtech II) of fruit at harvest (Diamond symbols) and 28 days post-harvest (Square symbols). Each symbol represents an average of five replicates, each comprising 15 fruit from two branches. Each symbol was from separate treatments of thinned to 2 or 4 buds per spur or left with the natural crop load at pre-bloom or 6 weeks after full bloom, i.e. three thinning treatments x 2 thinning times = 6 treatments. $y=0.0004x-0.0416$, $R^2=0.9244$, $p<0.0001$

4. Discussion

This study reveals significant interaction between crop load and the time of thinning on sweet cherry fruit quality. High crop loads resulted in significant reductions to fruit size in 'Sweetheart' when thinned several weeks after flowering (i.e., 6 and 8 WAFB), but there was no loss of size with earlier thinning. Further, these late thinning times resulted in significant decreases in firmness and TSS under higher crop loads in 'Sweetheart'. The reductions in TA of fruit from trees thinned after FB were not observed in fruit from trees thinned earlier. These findings are consistent with earlier reports that supply of carbohydrates and nutrient resources is limited under high crop load (Proebsting & Mills 1981). This may be particularly so for 'Sweetheart' trees trained to the KGB system and thinned after full bloom, presumably due to greater investment of carbohydrate resources in fruitlets that were subsequently thinned. This suggestion is supported by our data on tree carbohydrate reserves that reveal significantly lower levels in limbs thinned 8 WAFB compared with those thinned while dormant. However, replication of the impacts seen in this study on 'Sweetheart' would be required to show that they are consistent. The results for 'Van' in 2010/11 were in contrast to the results found in 'Sweetheart'. Studies by Einhorn et al. (2011) and Measham et al. (2012) have shown that manipulation of crop load has had inconsistent results over different seasons. A general decline in fruit size was observed with time of thinning from FB irrespective of crop load treatment and no effects of timing of thinning on firmness, TSS or TA were observed in Van in 2010/11. The reasons for the difference in response between varieties in 2010/11 can be speculated to be variety or age-related differences, although crop load differences can be eliminated as the treatments arrived at virtually identical crop loads between the varieties.

The results of this study may imply a distinct carbohydrate source:sink relationship in 'Sweetheart' relative to 'Van', where carbohydrate source is limiting potential fruit size and earlier thinning results in quality benefits such as increased size – perhaps due to greater resource availability for cell division given that cell division is complete within 10 days of full bloom (Tukey & Young 1939) and that no similar decline was seen in firmness, TSS or TA. That potential fruit size is set early in the fruit development ontogeny is tacitly supported by the lack of considerable difference in size between the 1 and 2 bud/spur treatments in either variety whilst firmness was significantly higher in the 1 than the 2 bud/spur treatments in both varieties in one season. This is additionally supported by an earlier study (Measham et al., 2012) that suggested pre-bloom thinning would be more effective for manipulating size whilst minimizing the risk of fruit cracking through water uptake during cell expansion in the later stages of fruit growth.

This study observed distinct impacts of crop load manipulation in 'Van' over two seasons. The impacts of crop load on fruit quality were only significant in the first season, when the natural crop load was generally higher than the second season. This is consistent with other studies that suggest crop load manipulation as a means to improve fruit quality is neither consistent, nor necessary, unless a high natural crop load occurs (Neilsen et al., 2007, Einhorn et al., 20011; Measham et al., 2012). It should be noted that crop loads in all trials in this study range from medium to light according to the criteria set by Neilsen et al. (2007), however these values were set for fruit grown on 'Gisela 5' rootstock which is precocious compared to F-12/1 (Robinson and Hoying in press). These authors suggest that 400 g fruit/cm^2 trunk cross-sectional area (or approx. 45 fruit/cm^2 TCSA) represents a high crop load for Lapins, while 100 g fruit/cm^2 trunk cross-sectional (or approx. 10 fruit/cm^2 TCSA) area is a high load for 'Bing, and that high crop load thresholds therefore vary with variety. Measham et al. (2012) did not find a fruit size diminishing effect in trees grown on Mazzard until a threshold of 25 fruit/cm^2 TCSA was achieved, and that the risk of cracking increased below a threshold of 10 fruit/cm^2 TCSA in several varieties. Moreover, in manipulated crop load trials (Measham et al. in press) and in a survey of natural crop load (Measham et al. 2012) of varieties grown in Southern Tasmania, loads rarely exceed 15 fruit/cm^2 TCSA. Regulating crop load for optimum size therefore, relies on the knowledge of crop load thresholds at which size diminishes for each variety/rootstock combination. In these previous studies and in the second year of this current study, the levels may not have reached that threshold.

Negative correlations of all quality parameters, except colour, were found with increasing crop load. This emphasizes the need for effective crop load monitoring, with regulation as needed, particularly where high quality of fruit is necessary for successful export. Whilst most quality measure, comparisons within the 2010/11 season were similar for a given crop load and time of thinning, flesh firmness was higher for a given crop load in 'Sweetheart' than 'Van'. Olmstead et al. (2007) reported differences between cultivars in fruit mesocarp cell numbers, hence the difference in firmness observed between 'Sweetheart' and 'Van' fruit in this study may be attributable to differences in cell numbers between the two cultivars. The two varieties also have different ripening periods such that cell expansion and water content could be a contributing factor.

Strong correlations were found between firmness measured with a FirmTech compression test and either a skin or flesh penetration test measured with a Güss texture analyser, irrespective of whether fruit was measured at harvest or 28 days post-harvest. This is despite the skin puncture measure including force required to puncture relatively 'elastic' skin that may not reflect flesh firmness (Looney & Webster 1996). These correlations suggest that for practical purposes, measurement of firmness with the relatively quick Firmtech can replace either flesh firmness or skin puncture force penetrometer measurements. Additionally, results can be easily assimilated by industry stakeholders as it is a widely used tool in processing houses.

Post-harvest fruit quality assessments of 'Van' fruit showed an increase in all measures of firmness due to dehydration, consistent with the decrease in fruit weight (Drake & Elfving, 2002; Alique et al., 2006; Puniran et al., 2012). Whilst crop load affected sugars and TA at harvest, treatments responded similarly post-harvest, emphasizing the importance of initial quality to the consumer experience post-harvest. This is consistent with the conclusion of Kupferman (1986) that the best time to harvest is at Mahogony stage when firmness, weight and TSS are highest. However, according to Drake and Elfving (2002), while skin colour has long been accepted as the best indicator for the appropriate harvest maturity, loss of quality after storage for 14 days or more is higher for fruit harvested at normal commercial time or later compared with harvesting up to 5 days earlier. Hence there is a risk in harvesting too mature and allowing acidity to drop which has significant implications for flavour perception.

5. Conclusion

The bud thinning technique employed resulted in a wide spread of crop loads, and is therefore superior to the use of ATS sprays (eg. Schoedl et al., 2009) for the purpose of investigating fruit quality and carbohydrate storage responses to crop load regulation, particularly as all chemical thinning agents can impact negatively on fruit quality (Bound, 2001).

This study showed that crop load and timing of thinning interact – particularly for fruit produced using the KGB system. Results indicated that internal carbohydrate reserves were significantly depleted in late-thinned limbs, although it must be cautioned that these were only investigated in one season. Generally, sweet cherry fruit quality (size, firmness, sugar, acid and stem retention) was optimised through attaining crop load of approximately 10 fruit per cm^2 limb cross-sectional area through thinning at dormancy or full bloom in this study, although it must be cautioned that poor cracking outcomes have been observed when thinned at full bloom (Measham et al., 2012). It is worth noting though that desired crop loads that optimise fruit quality at harvest can only be achieved by thinning if natural load is high, but that diameter increases of 2-3 mm in response to thinning can increase fruit value by

$2-3/kg if it results in 'up-sizing' to 28 mm+ or 30 mm+ size categories (Reid Pers. Comm. May 2013). Additionally, achieving quality from low loads must be balanced with the risk of increased cracking (Measham et al., 2012) and in some cases reduced yields (Einhorn et al., 2011) or very large soft fruit (Neilsen et al., 2007). The development of a 'user-friendly' guide for growers to assess fruit set and subsequent crop load quickly would be beneficial in decision making processes around thinning for optimal fruit quality.

The similar rates of deterioration in post-harvest quality regardless of crop load or timing of thinning *per se*, emphasises the importance of fruit quality at-harvest to ensure post-harvest quality of exported fruit for the consumer. This study exposes the interactive effects of the level and timing of crop-load regulation on cherry fruit quality. Alternative growing systems may result in more effective light capture and therefore enable the production of higher yields, without a cost to quality, than that currently achieved under the KGB system.

Acknowledgements

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The Effect of Resveratrol on the Quality of Extended Boar Semen During Storage at 17ºC

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Abstract

The natural polyphenol resveratrol may be beneficial to many aspects of cell function and animal health, although its actions in the male reproductive system vary depending on animal species. This work investigates resveratrol effects on the quality of preserved boar semen during liquid storage at 17ºC. We used three approaches: 1) evaluation of conventional parameters of seminal quality, 2) measurement of specific response to capacitating stimuli, and 3) evaluation of mitochondria membrane potential and ATP content. Resveratrol supplementation causes i) a loss in the response of liquid stored boar spermatozoa to capacitating stimuli, ii) a decrease in the sperm ATP content and iii) a reduction in the mitochondrial membrane potential. Moreover, higher concentrations of resveratrol increase plasma membrane phospholipid disorder and reduce the percentage of motile spermatozoa. These results suggest that semen doses supplemented with resveratrol could be considered sub-fertile compared with semen stored hypothermically in standard conditions.

Keywords: resveratrol, boar semen storage, calcium influx, ATP

1. Introduction

Nowadays in the worldwide pig production, artificial insemination (AI) is made with semen that has been extended in the liquid state and stored at 15-20ºC for 1 to 5 days (Johnson et al., 2000). AI allows a better distribution of genetic material of high quality and minimizes boar transportation. To preserve spermatozoa for prolonged periods, their metabolic activity needs to be reduced and this is approached by semen dilution into an appropriate medium and by lowering the temperature. Several commercial boar extenders have been proposed with this objective (Gadea, 2003), although the most widely used extender is the Beltsville-Thawing Solution (BTS) developed by Pursel and Johnson (1975) for thawing boar spermatozoa frozen in the pellet form, and later adapted for liquid storage (Pursel et al., 1978). Sperm membrane is rich in polyunsaturated fatty acid, which makes them very susceptible to oxygen-induced damage mediated by lipid peroxidation (LPO), especially in boar spermatozoa, which contains a high concentration of polyunsaturated fatty acids (Awda et al., 2009; Waterhouse et al., 2004). Several antioxidants have been used successfully in the supplementation of boars extenders: superoxide dismutase and catalase (Roca et al., 2005), α-tocopherol (Cerolini et al., 2000) and L-Glutamine (Funahashi & Sano, 2005).

Resveratrol (RSV) is a natural grape-derived polyphenolic phytoalexin that possesses pleiotropic effects including anticancer, anti-aging, anti-inflammatory and anti-oxidant actions, as well as cardioprotection and neuroprotection (Fulda et al., 2010; Pervaiz & Holme, 2009). RSV effectively scavenges superoxide and peroxynitrite radicals generated from enzymatic and non-enzymatic systems, and afford protection against DNA damage caused by reactive oxygen species (ROS) in somatic cells (K. W. Lee & H. J. Lee, 2006).

Effects of resveratrol in spermatozoa have been studied in recent works. Thus, RSV addition (0.1, 1.0 and 10.0 mM) to cryopreservation medium of human spermatozoa is able to prevent cryopreservation-induced lipid damage (Garcez et al., 2010) and at 10 mM also the cryopreservation-induced DNA damage (Branco et al., 2010). Although in both studies RSV treatment is not able to prevent the observed reduction in sperm motility after thawing, they conclude that this polyphenol might be used for the process of cryopreservation of human spermatozoa, at least for intracytoplasmic sperm injection (ICSI) where motile spermatozoa are not needed to achieve fertilization.

In a different specie, ram, the use of RSV in the cryopreservation medium causes a decrease in the mitochondrial membrane potential without any effects in spermatozoa motility, or the integrity of acrosome or plasma membrane (Silva et al., 2012).

Collodel et al. (2011) describe that 100 µM resveratrol treatment leads to a loss in viability in human spermatozoa as well as in rat spermatocytes, although at 15 µM has a protector effect against ROS.

Resveratrol has been also studied as an antioxidant added to the diet of rats (Juan et al., 2005), where results are beneficial. The lower diameter of seminiferous tubules together with an increase in the density of testicular tubules produces an increase in the spermatogenic tissue would explain the increase in the spermatozoa concentration observed in rats supplemented with 20 mg/Kg resveratrol in their diets.

A recent investigation showed that intraperitoneal administration of resveratrol to rats prevents the loss of sperm motility, leads to a decrease in lipid peroxidation and prevents against oxidative stress occurring in rats with hyperthyroidism (Ourique et al., 2013).

Based on the beneficial effects of RSV in different species, the objective of the present work is to study the use of resveratrol to improve the storage of boar seminal doses at 17ºC. Therefore, we have used different experimental approaches: 1) Evaluation of conventional sperm parameters to evaluate quality of seminal doses; 2) Evaluation of calcium influx and the specific sperm response to bicarbonate as proposed by Harrison et al. (1993), and 3) Analysis of resveratrol effects on sperm mitochondria membrane potential and ATP concentration.

2. Materials and Methods:

2.1 General Experiments Design

2.1.1 Experimental Design 1

A total of 12 ejaculates (6 males, 2 ejaculates each) were diluted in BTS at final concentration of 35 x 10^6 spermatozoa/mL and subsequently analyzed (day 0). Semen samples were treated without or with different concentrations of resveratrol: 10, 33, 66 and 100 µM or DMSO (0.14 %) and preserved at 17ºC for several days. Motility parameters as well as flow cytometry analyzed parameters (sperm viability, plasma membrane phospholipid disorder, acrosome membrane integrity and mitochondrial membrane potential) were analyzed at days 1, 4 and 7 of preservation.

2.1.2 Experimental Design 2

This experiment is aimed to analyze the calcium influx kinetics and specific response to capacitating conditions in spermatozoa stored at 17ºC in BTS without or with RSV at days 1, 4, and 7 of storage. A total of 8 ejaculates (n=8) from 8 different animals were diluted in BTS at final concentration of 20 x 10^6 spermatozoa/mL and 33 and 100 µM RSV were added. The specific response to bicarbonate under capacitating conditions in vitro was assessed by monitoring the plasma membrane integrity and intracellular calcium concentrations after incubation of spermatozoa for 3 and 60 min.

2.2 Chemicals and Sources

Beltsville-Thawing Solution was obtained from Minitüb GmbH (Tiefenbach, Germany). Live/dead spermatozoa viability kit including both propidium iodide (PI) and SYBR-14 probes, M540 and YoPro-1 probes were purchased from Molecular Probes (Leiden, The Netherlands). Resveratrol (3,4′,5-Trihydroxy-trans-stilbene), ATP kit (FL-AA), phosphatase inhibitor cocktail 2 (P5726) and FITC-PNA were from Sigma-Aldrich® (St Louis, MI, USA); coulter isoton II diluent was from Beckman Coulter Inc. (Brea, CA, USA). JC-1 (5,5′,6,6′–tetrachloro-1,1′,3,3′tetraethylbenzymidazolyl carbocyanine iodine) probe was purchased from Life Technologies Ltd (Grand Island, NY, USA), Fluo-3/AM from Axxora (Lörrach, Germany), 96 well plates (clear bottom, no. 655088) from Greiner bio-one, and Percoll®-saline from GE Healthcare (Munich, Germany). The rest of the chemicals were purchased from Merck (Darmstadt, Germany) and Roth (Karlsruhe, Germany).

2.3 Animals, Sample Collection and Semen Preparation

Fourteen boars (Duroc, Pietrain and German Large White breed, 2-4 years of age) housed at a commercial insemination station (Tecnogenext, S.L (Spain)) and at the Unit for Reproductive Medicine of Clinics

(University of Veterinary Medicine Hannover, Germany) were used as ejaculate donors. Artificial insemination using liquid preserved semen from these boars demonstrated their fertility. Fresh ejaculates were collected with the gloved hand technique and immediately placed in a water bath at 37ºC. After collection, a computer-assisted sperm analysis system (CASA) (ISAS Psus®, Proiser R+D S.L., Paterna, Valencia) was used for the evaluation of sperm concentration and motility. Morphology was evaluated by eosin-nigrosin staining, a total of 200 spermatozoa per ejaculate were evaluated by oil immersion microscopy objective 100X. Only ejaculates with at least 80% morphologically normal spermatozoa, 70% motile spermatozoa and a total number of spermatozoa higher than 10 x 10^9 were used. Immediately after collection, sub-samples of each ejaculate were diluted in BTS extender (Minitüb GmbH, Germany) and treated as follows: a) supplementation with different concentrations of resveratrol (70 mM stock solution in dimethyl sulfoxide, DMSO), b) addition of the highest DMSO concentration (0.14 %) used to supplement resveratrol, and c) untreated sub-sample (BTS). Sperm sub-samples were cooled at room temperature for 1.5 h and subsequently stored in a refrigerated incubator (FOC 225 I, VELP Scientifica, Usmate, Italy) at 17ºC in sealed containers excluding air, in an anaerobic medium, for seven days.

2.4 Assessment of Spermatozoa Motility

Prior to motility analysis, seminal doses (500 µL) were incubated for 40min with 5 % CO_2 at 38.5ºC (Mini Galaxy A, RS Biotech, United Kingdom). After gentle mixing, semen was examined for motility pattern using a CASA system (ISAS® program, Proiser R+D, Paterna, Valencia, Spain) following the manufacture's guidelines. A total of 2 µL of sample were placed in a pre-warmed counting chamber (Leja®, Nieuw-Vennep. The Netherlands). At least 300 spermatozoa per sample were analyzed and the following sperm motility parameters were recorded: total motile spermatozoa (percentage of spermatozoa with an average path velocity > 10 µM/s), progressive motile spermatozoa (percentage of spermatozoa with a straightness coefficient > 80 %), VCL (curvilinear velocity in µM/s), VSL (straight-line velocity in µM/s), VAP (average path velocity in µM/s), LIN (linearity coefficient in %) and STR (straightness coefficient in %).

Examinations at the University for Veterinary Medicine in Hannover were carried out with SpermVision® program as described in Henning et al. (2012). A 0.63 camera adapter (U-PMTVC tv-0.63, Olympus, Hamburg, Germany) was used.

2.5 Flow Cytometry Analysis

In experiment one, a Coulter EPIC XL flow cytometer (Beckman Coulter Ltd.) was used to evaluate sperm viability, plasma membrane phospholipid disorder, acrosome membrane integrity and mitochondrial membrane potential and data were analyzed using a FACStation™ and EXPOTM 32 ADC software (Beckman Coulter, Inc.). Fluorophores were excited by a 15 mW argon ion laser operating at 488 nm. A total of 10,000 gated events (bases on the forward scatter and side scatter of the sperm population recorded in the linear mode) were collected per sample with a sample running rate of approximately 500 events/sec. Fluorescence data were collected in the logarithmic mode.

In experiment two, a DAKO Galaxy flow cytometer (DAKO, Hamburg, Germany) controlled by FloMax software (version 2.4, Partec, Münster, Germany) was used for intracellular calcium analysis (Hoechst 33342, PI, Fluo-3) and assessment of plasma and acrosomal membrane integrity (Hoechst 33342, PI, FITC-PNA). The cells were excited at two spots while passing the cuvette: first at a wavelength of 488 nm (argon ion laser; 20 mW) and second at a wavelength of 365 nm (mercury lamp; 100 W). Fluorescence signals were detected using a 455/10 band pass filter (Hoechst 33342), a 537.5/22.5 nm bandpass filter (Fluo-3, FITC-PNA), and a 630 nm long pass filter (PI). A logical gate was used to identify the sperm population based on positive staining for Hoechst 33342 (DNA-containing events) and on being in the expected size range for spermatozoa in the forward scatter distribution. A total of 10,000 events fitting the definition of the logical gate were counted. The overlap of spectra between PI and Fluo-3 or FITC-PNA was compensated post acquisition.

2.6 Assessment of Spermatozoa Viability

As described previously (Martin-Hidalgo et al., 2011), fluorescent staining using the LIVE/DEAD Sperm Viability Kit was performed to assess porcine spermatozoa viability. Briefly, 100 µL of liquid preserved semen (35 x 10^6 cells/mL) was diluted with 400 µL isotonic buffer (coulter isoton II). 5 µL of SYBR-14 (2 µM final concentration) and 10 µL of propidium iodide (5 µM final concentration) were added to 500 µL of the diluted sample and incubated for 20 min at room temperature in the darkness. After incubation, cells were analyzed by flow cytometry and the percentage of viable spermatozoa (SYBR14-positive and PI-negative) was recorded.

2.7 Assessment of Spermatozoa Plasma Membrane Phospholipid Disorder

Changes in membrane phospholipid disorder were assessed by using a merocyanine 540 (M540) and YoPro-1 double staining. Aliquots of 100 µL of each semen sample (35 x 10^6 cells/mL) were diluted in 400 µL of isotonic buffered diluent containing 2 µL of YoPro-1 (0.08 µM final concentration), then mixed and incubated at 38ºC for 15 min. Just before analysis, 2 µL of M540 (4 µM final concentration) was added to each sample and incubated for 2 min and mixed before flow cytometry analysis. Labeled spermatozoa were categorized as (1) viable cells with low plasma membrane phospholipid disorder (YoPro-1^-/M540^-); (2) viable cells with high plasma membrane phospholipid disorder (YoPro-1^-/M540^+); or (3) non-viable cells with altered permeability of the plasma membrane (Yo-Pro-1^+).

2.8 Assessment of Spermatozoa Acrosome Integrity

The acrosomal status of spermatozoa was assessed after staining the spermatozoa with fluorescein-isothiocyanate conjugated peanut agglutinin (PNA-FITC), as a marker for acrosome status, and PI. Aliquots of 100 µL of each semen sample (35 x 10^6 cells/mL) were incubated at room temperature in the dark for 5 min with 5 µL of PNA-FITC stock solution (3 µg/mL in DMSO) and 5 µL of PI (6 µM final concentration). Just before flow cytometry analysis, 400 µL of isotonic buffered diluent was added to each sample. Cells were analyzed and the percentage of live spermatozoa with damaged or reacted acrosome (PI-negative and PNA-positive spermatozoa) was recorded.

2.9 Assessment of Spermatozoa Mitochondrial Membrane Potential

Mitochondrial membrane potential variations were evaluated using the probe JC-1. This lipophilic cationic fluorochrome JC-1 is present as protomeric aggregates in mitochondria with high membrane potential and emits light in the orange spectrum (590 nm) when excited at 488 nm. In mitochondria with low membrane potential, JC-1 is present as monomers that emit light in the green spectrum (525 nm). From each sperm sample, 100 µL (35 x 10^6 cells/mL) were diluted in 400 µL of isotonic buffered diluent containing 3 µL of JC-1 (1 µM final concentration) and then mixed and incubated at 38ºC for 30 min. The samples were mixed before flow cytometry analysis. The percentage of orange stained cells, which represents the population of male germ cells with high mitochondrial membrane potential (hMMP), was recorded.

2.10 Assessment of Calcium Influx in Spermatozoa

Calcium influx and the specific response to bicarbonate in liquid preserved boar spermatozoa were assessed as described in Henning et al. (2012) with minor modifications. Three types of a Tyrode's medium were used for exposing spermatozoa to capacitating or non-capacitating conditions. The complete Tyrode's medium or TyrBicCa consisted of 96 mM NaCl, 20 mM HEPES, 5 mM glucose, 3.1 mM KCl, 0.4 mM $MgSO_4$, 0.3 mM KH_2PO_4, 100 µg/mL gentamycin sulfate (SERVA, Heidelberg, Germany), 20 µg/mL phenol red, 1.0 mM sodium pyruvate, 21.7 mM sodium lactate, 3 mg/mL bovine serum albumin (Cohn's fraction V, fatty acid free), 15 mM $NaHCO_3$ and 2 mM $CaCl_2$. In the non-capacitating control media, either bicarbonate (TyrCa) or both, bicarbonate and $CaCl_2$ (TyrControl), were omitted. In TyrControl 1 mM Na_2-EGTA (disodium ethylene glycol tetracetate) was added. All media were adjusted to a pH of 7.4 at 38ºC and an osmolality of 300±5 mOsmol/kg. For equilibration, TyrBicCa was kept in an incubator (38°C) under 5% CO_2 and 100% humidity, whereas TyrCa and TyrControl were kept sealed in a heating cabinet (38°C).

Spermatozoa were prepared by adding 2 µL of a Fluo-3/AM stock solution (1 mM in DMSO) to 2 mL of sperm suspensions (20 x 10^6 spermatozoa/mL) and incubated in the dark at room temperature. After 30 min, spermatozoa were centrifuged through a discontinuous gradient of 35 and 70% iso-osmotic Percoll® saline essentially as described by Harrison et al. (1993). Spermatozoa were layered over a two-step gradient of 4 mL of 35 % Percoll-saline on 2 mL of 70% Percoll-saline. Tubes were centrifuged at 300 g for 10 min followed by 15 min at 750 g. After centrifugation, the supernatant was aspirated and sperm pellet was resuspended in TyrControl without RSV and BSA. The suspension of Fluo-3 loaded and washed sperm was kept in the dark at ambient temperature and used within 30 min of preparation. Aliquots of 5 µL of Fluo-3-loaded sperm were diluted in 995 µL of TyrBicCa, TyrCa or TyrControl medium without RSV added and supplemented with PI (final concentration 2.5 µg/mL) and Hoechst 33342 (final concentration 0.75 µg/mL). Samples were analyzed after 3 and 60 min on the DAKO Galaxy flow cytometer. Signals for PI distinguished between death cells with defective plasma membranes (PI-positive) and live cells with intact plasma membranes (PI-negative), whereas Fluo-3 signal subdivided the PI-negative spermatozoa population into cells with a low Fluo-3 fluorescence signal (live, low-Ca^{2+} sperm cells; Fluo-3-negative) and those with a higher Fluo-3 fluorescence signal (live, high-Ca^{2+} sperm cells; Fluo-3-positive). The change in the amount of a spermatozoa subpopulation between 3 and 60 min of incubation in TyrBicCa, TyrCa or TyrControl medium indicates the responsiveness of a sperm sample to capacitating conditions (Henning et al., 2012; Schmid et al., 2013). Responsiveness was calculated as changes in the live, low- Ca^{2+} subpopulation (PI-negative/ Fluo-3-negative; Δ = 60 min-3 min). The specific response to bicarbonate upon exposure of spermatozoa to capacitating conditions was calculated as the difference in the responsiveness to incubation conditions in TyrBicCa (Δ60-3) and TyrCa (Δ60-3) as described in Schmid et al. (2013).

2.11 Quantification of ATP Spermatozoa Content

ATP content of spermatozoa was quantified at storage temperature as well as after incubation at 38°C. The assessments were done according to Long and Guthrie (2006) with minor modifications. Immediately after removal from the refrigerated incubator, a total of 100 µL of stored semen (20 x 10^6 spermatozoa/mL) was mixed with 1 µL of phosphatase inhibitors. Another 100 µL of stored semen were incubated with 5 % CO_2 at 38.5ºC for 40 min and then 1 µL of phosphatase inhibitors was added. After 30 min incubation with inhibitor at room temperature, spermatozoa were frozen and stored at -20ºC. For ATP extraction, samples were boiled for 10 min with 900 µL of boiling buffer (50 mM Tricine, 10 mM $MgSO_4$, 2 mM EDTA, pH= 7.8) and then chilled on ice for 10 min and centrifuged at 5,000 g for 30 min at 4 °C. ATP content was measured in 25 µL of the supernatant using the ATP bioluminescent assay kit (FL-AA) from Sigma-Aldrich® following the manufacturer's guidelines. The 96 well microtiter plates were measured in a SpectraFluor Plus plate reader (Tecan Group Ltd., Maennedorf, Switzerland).

2.12 Statistical Analysis

A power analysis was conducted to determine the appropriate number of animals and samples. The mean and standard error of the mean were calculated for descriptive analysis. Q-Q plots were used to check for departures from the normal distribution. All the sperm variables satisfied the normality requirement for a parametric analysis of variance.

The effects of treatment (untreated and Resveratrol) and storage time (1, 4 and 7 days) on several seminal characteristics (motility, ATP content, viability, plasma membrane phospholipid disorder, acrosomal membrane integrity, mitochondrial membrane status and intracellular calcium content) were analyzed statistically using a General Linear Model, mixed-effects model (with boars and ejaculates within boars as random effects and treatment and storage time as fixed effects) was applied to the experimental design. Bonferroni's test was used to perform post hoc tests. The Pearson's correlation test was used to study the correlation among the quantity of ATP with different motility parameters (percentage of motile spermatozoa, VCL, VAP and VSL) and resveratrol concentration used. All analyses were performed using SPSS v15.0 for Windows software (SPSS Inc. Chicago, IL). The level of significance was set at $p < 0.05$, except for the study of correlation where the level of significance was set at $p < 0.01$.

3. Results

In this work, the possible effect of solvent DMSO (0.14 %) was tested for all investigated parameters and no effect was observed compared with semen stored in BTS alone (data not shown).

3.1 Effect of Resveratrol on Spermatozoa Motility During Boar Semen Storage at 17ºC in BTS

To evaluate the effect of RSV in spermatozoa motility parameters during semen storage, boar seminal doses were diluted in BTS without or with different concentrations of RSV (10, 33, 66 and 100 µM). After BTS dilution (day 0) and after 1, 4 and 7 days of storage at 17ºC, seminal doses were incubated with 5% CO_2 at 38.5ºC during 40 min to ensure motility and then motility parameters were evaluated (experimental design 1).

At low doses (10 and 33 µM) RSV addition was without effect in the percentage of motile spermatozoa (Table 1). However, addition of higher doses of RSV (66 and 100 µM) caused a statistically significant reduction in the percentage of motile spermatozoa starting at 4 days of storage (Table 1). A similar RSV effect was observed in the velocity parameters (Table 1) with a clear and statistically significant reduction in VCL (Table 1), VAP and VSL (data not shown) in spermatozoa stored in presence of 66 and 100 µM of RSV.

Addition of RSV was without any effect in both the linearity of spermatozoa movement, as well as in the percentage of spermatozoa showing progressive movement at any dose or storage time (Table 2).

Table 1. Spermatozoa motility parameters after addition of different resveratrol concentrations to boar semen doses preserved at 17ºC for 7 days

	MOTILE SPERMATOZOA (%)				CURVILINEAR VELOCITY (μm/s)			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
BTS		91.0±1.7^{a,1}	89.8±1.5^{ab,1}	79.1±3.2^{b,1}		76.4±4.85^{a,1}	78.1±4.2^{a,1}	75.4±4.7^{a,1}
RSV10		91.0±2.4^{a,1}	88.5±2.2^{ab,1}	79.8±3.7^{b,1}		76.6±4.7^{a,1}	72.2±4.1^{a,1}	76.3±4.3^{a,1}
RSV33	93.4±1.7^{a}	91.3±2.3^{a,1}	86.3±2.0^{a,1}	72.9±5.4^{b,1}	74.6±5.4^{a}	78.3±4.7^{a,1}	70.4±4.1^{a,1}	65.6±4.6^{a,1,2}
RSV66		86.8±3.3^{a,b,1}	80.6±2.7^{b,1,2}	60.4±6.1^{c,2}		76.9±3.7^{a,1}	62.6±3.5^{a,1,2}	53.5±3.9^{b,2,3}
RSV100		83.1±3.6^{a,b,1}	71.9±4.7^{b,2}	51.2±5.7^{c,2}		64.3±3.6^{a,1}	47.3±4.7^{b,2}	44.3±3.2^{b,3}

Boar seminal doses were preserved at 17ºC during 7 days in BTS in absence or presence of different concentrations of RSV (10, 33, 66 and 100µM). Percentage of motile spermatozoa and curvilinear spermatozoa velocity (VCL in µm/s) were measured by CASA system (ISAS®) as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM) (n=12). Within a parameter (column) or within a RSV concentration given (row), different superscripts (^{a,b,c}) mean statistical differences between days. Within a parameter and for a given day of preservation, differences between RSV concentrations are indicated by different numerical superscript ($^{1, 2, 3}$).

Table 2. Spermatozoa motility parameters after addition of different resveratrol concentrations to boar semen doses preserved at 17ºC for 7 days.

	PROGRESSIVE MOTILITY (%)				LINEARITY INDEX (%)			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
BTS	52.8±3.4^{a}	55.5±3.0^{a,1}	56.8±4.0^{a,1}	47.6±3.3^{a,1}	61.3±1.6^{a}	61.9±1.6^{a,1}	61.8±2.0^{a,1}	56.1±1.9^{a,1}
RSV10		58.1±2.9^{a,1}	56.0±3.0^{a,1}	48.8±3.4^{a,1}		62.8±1.5^{a,1}	61.5±1.6^{a,1}	56.7±1.8^{a,1}
RSV33		54.8 ±3.3^{a,1}	58.1±3.6^{a,1}	51.3±1.6^{a,1}		61.0±1.6^{a,1}	61.3±2.0^{a,1}	56.5±0.9^{a,1}
RSV66		54.5±3.1^{a,1}	61.0±3.2^{a,1}	51.2±1.8^{a,1}		59.0±1.7^{a,b,1}	60.9±1.8 ^{a,1}	54.2±1.3^{b,1}
RSV100		58.6±2.0^{a,1}	57.9±3.5^{a,1}	48.2±1.6^{a,1}		60.0±1.3^{a,1}	57.1±2.0^{a,1}	51.0±1.1 ^{b,1}

Boar seminal doses were preserved at 17ºC during 7 days in BTS in absence or presence of different concentrations of RSV (10, 33, 66 and 100µM). Progressive motility and linearity index was measured by CASA system (ISAS®) as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM) (n=12). Within a parameter (column) or within a RSV concentration given (row), different superscripts (^{a,b,c}) mean statistical differences between days. Within a parameter and for a given day of preservation, differences between RSV concentrations are indicated by different numerical superscript ($^{1, 2, 3}$).

Table 3. Spermatozoa viability and the integrity of acrosome and plasma membranes after addition of different resveratrol concentrations to boar semen doses preserved at 17ºC for 7 days

	CELL VIABILITY (SYBR-14^{+}/PI^{-})				ACROSOME INTEGRITY (PNA^{+}/PI^{-})				PLASMA MEMBRANE PHOSPHOLIPID DISORDER (M540^{+}/YOPRO^{-})			
	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7	Day 0	Day 1	Day 4	Day 7
BTS	93.4±1.7^{a}	92.6 ± 0.5^{a,1}	91.8 ± 0.8^{a,1}	89.2 ± 1.3^{a,1}	3.7±0.5^{a}	4.2±0.7^{a,1}	4.9±0.8^{a,1}	8.1±1.7^{a,1}	8.6±0.8^{a}	8.8±0.6^{a,1}	15.0±2.9^{a,b,1}	22.0±4.1^{b,1}
RSV10		91.5± 0.6^{a,1}	89.2± 1.1^{a,1}	88.7± 0.8^{a,1}		4.0±0.5^{a,1}	5.7±1.0^{a,b,1}	9.4±2.5^{b,1}		10.5±0.7^{a,1}	14.2±2.9^{a,b,1}	21.1±4.7^{b,1}
RSV33		91.7± 0.5^{a,1}	88.9± 1.0^{a,1}	88.8± 0.8^{a,1}		4.0±0.5^{a,1}	5.2±0.7^{a,b,1}	9.2±2.2^{b,1}		10.1±0.8 ^{a,1}	15.9±3.3^{a,1}	25.9±5.8^{b,1}
RSV66		91.3 ± 0.4^{a,1}	89.5 ± 1.0^{a,1}	88.1 ± 1.0^{a,1}		4.0±0.4^{a,1}	5.6±0.8^{a,b,1}	10.8±2.7^{b,1}		10.5±0.8^{a,b,1}	20.0±4.2^{b,c,1}	28.3±5.8^{c,1}
RSV100		91.5± 0.7^{a,1}	89.8± 1.1^{a,1}	86.1± 1.7^{a,1}		4.2±0.7^{a,1}	4.9±0.8^{a,1}	12.3±2.9^{b,1}		9.9±0.8^{a,1}	22.8±4.6^{b,1}	41.6±6.1^{c,2}

Boar seminal doses were preserved at 17ºC during 7 days in BTS in absence or presence of different concentrations of RSV (10, 33, 66 and 100 µM). Percentages of cell viability, plasma membrane phospholipid disorder and acrosomal integrity of spermatozoa were measured by flow cytometry as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM) (n=12). Within a RSV concentration given (row), differences between days for a given parameter (column) are indicated by different superscripts (^{a,b,c}).

Within a parameter and for a given day of preservation (column), differences between RSV concentrations are indicated by different numerical superscript ([1, 2, 3]).

3.2 Effects of Resveratrol on the Viability, Plasma Membrane Phospholipid Disorder and Acrosome Membrane Integrity of Spermatozoa During Boar Semen Storage at 17ºC in BTS

To evaluate the effect of RSV in spermatozoa viability, plasma membrane phospholipid disorder and acrosome membrane integrity during semen storage we used the experimental design 1 described in materials and methods.

The storage of boar semen in the presence of different doses of RSV (10, 33, 66 and 100 µM) did not affect either the percentage of viable spermatozoa with an intact plasma membrane or the percentage of spermatozoa presenting altered acrosomal membrane, compared with BTS alone at any storage time or dose (Table 3).

Only the highest doses of RSV used (100 µM) caused a statistically significant increase in the percentage of spermatozoa showing a higher disorder on plasma membrane phospholipid on day 7 of semen storage (Table 3).

3.3 Calcium Influx and the Specific Response to Bicarbonate in Boar Semen Stored at 17ºC in BTS in Presence of RSV

To evaluate the effect of RSV on calcium influx and the specific response of boar spermatozoa to bicarbonate, boar seminal doses were diluted in BTS without or with different concentrations of RSV (33 and 100 µM). At 1, 4 and 7 days of storage at 17ºC, specific response to bicarbonate upon exposure of spermatozoa to capacitating conditions was calculated as the difference in the responsiveness to incubation conditions in TyrBicCa (Δ60-3) and TyrCa (Δ60-3). Only boar seminal doses incubated with the highest dose of RSV show a statically significant reduction in the specific response to bicarbonate after 7 days of storage at 17ºC, with a 3-fold decrease compared with BTS alone (Table 4).

Table 4. Specific response to bicarbonate in boar semen doses supplemented with different resveratrol concentrations and preserved at 17ºC

	SPECIFIC RESPONSE BICARBONATE			**DESTABILIZING EFFECT OF EXTRACELLULAR CALCIUM**		
	Day 1	**Day 4**	**Day 7**	**Day 1**	**Day 4**	**Day 7**
BTS	43.9±4.1[a,1]	33.0±4.2[a,1]	21.5±4.2[b,1]	1.3±0.9[a,1]	3.7±1.4[a,1]	1.6±1.3[a,1]
RSV33	48.3±3.5[a,1]	39.7±2.2[a,1]	23.9±3.7[b,1]	3.8±1.7[a,1]	1.8±1.1[a,1]	3.5±2.9[a,1]
RSV100	44.3±6.4[a,1]	29.9±4.6[a,1]	6.8±5.2[b,2]	2.0±1.2[a,1]	-0.1±0.5[a,1]	11.5±4.0[b,2]

Responsiveness of spermatozoa was calculated as changes in the live, low- Ca^{2+} subpopulation (PI-negative/ Fluo-3-negative) at the beginning (3min) and after 60 min of incubation (Δ = 60 min-3 min) in TyrBicCa, TyrCa or TyrControl. The specific response to bicarbonate describes the difference in the responsiveness in TyrBicCa and TyrCa. The destabilizing effect of extracellular calcium was calculated as the difference in the responsiveness in TyrCa and TyrControl. Results are mean ± standard error of the mean (SEM) (n=8). Within a treatment (row), differences between days for a given spermatozoa response (column) are indicated by different superscripts ([a,b]). Within a given time (column), differences between RSV concentrations are indicated by different numerical superscript ([1, 2]).

In addition, the destabilizing effect that can be attributed to the presence of extracellular calcium was monitored as the difference in the responsiveness to incubation conditions in TyrCa and TyrControl. After 7 days storage at 17ºC, seminal doses stored in presence of RSV 100 µM showed a statistically significant increase in the amount of spermatozoa that destabilized due to the presence of extracellular calcium. Values were 7-fold higher when compared with BTS alone (Table 4).

3.4 Effects of Resveratrol on Mitochondrial Membrane Potential and ATP Content of Boar Semen Preserved at 17ºC in BTS

To evaluate the effect of RSV on mitochondrial membrane potential during semen storage, boar seminal doses were diluted in BTS without or with different concentrations of RSV (10, 33, 66 and 100 µM; experiment 1). After BTS dilution (day 0) and after 1, 4 and 7 days of semen storage at 17ºC, mitochondrial membrane potential of spermatozoa was evaluated. Supplementation with any dose of RSV causes a significant decrease in the percentage of spermatozoa displaying high MMP at any time of semen storage (Table 5), showing the higher and statistically significant effect at 1-day storage at 17ºC for any dose of RSV use (Table 5).

Table 5. Mitochondrial membrane potential after addition of different resveratrol concentrations to boar semen doses preserved at 17ºC for 7 days

	HIGH MITOCHONDRIAL MEMBRANE POTENTIAL (%)			
	Day 0	**Day 1**	**Day 4**	**Day 7**
BTS	73.5±2.6^{a}	65.8 ± 3.9^{a,1}	76.4± 2.8^{a,1}	76.2 ± 4.2^{a,1}
RSV10		35.7± 8.7^{b,2}	40.4± 11.1^{a,1}	44.9± 10.5^{a,2}
RSV33		38.3 ± 8.0^{b,c,1,2}	52,2 ± 6.8^{a,c,1}	50.0 ± 9.0^{a,b,1,2}
RSV66		32.4± 8.7^{b,2}	55.5± 6.1^{a,b,1}	59.3 ± 6.1^{a,b,1,2}
RSV100		31.7± 8.1^{b,c,2}	50.1± 8.1^{a,c,1}	62.0± 6.0^{a,1,2}

Boar seminal doses were preserved at 17ºC during 7 days in BTS in absence or presence of different concentrations of RSV (10, 33, 66 and 100µM). Percentage of spermatozoa with high mitochondrial membrane potential was measured by flow cytometry as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM) (n=12). Within a treatment (row), differences between days (column) are indicated by different superscripts (^{a,b,c}). Within a given time (column), differences between RSV concentrations are indicated by different numerical superscript (^{1,2,3}).

To evaluate the effect of RSV on ATP content during semen storage, boar seminal doses were diluted in BTS without or with different concentrations of RSV (33 and 100 µM; experiment 2). ATP content was determined directly from the stored samples (17°C) and after 40 min incubation at 38.5ºC under 5% CO_2 atmosphere to activate spermatozoa. Compared with BTS alone, both concentrations of RSV (33 and 100 µM) caused a clear and statistically significant decrease in the ATP content of spermatozoa at any time storage. The decrease in ATP content was present under unstimulated and stimulated conditions (Table 6). Analysis of data showed a statistically significant negative correlation between ATP concentration of spermatozoa and the dose of RSV used ($r=-0.767$, $p<0.01$). Moreover, further analysis of data from semen stored at 17ºC showed a significant positive correlation between the spermatozoa ATP content (Table 6) and the percentage of motile spermatozoa ($r=0.463$; $p<0.01$), VCL ($r=0.380$; $p<0.01$), VAP ($r=0.489$; $p<0.01$) and VSL ($r=0.557$; $p<0.01$).

Table 6. ATP levels in spermatozoa of boar semen doses supplemented with different resveratrol concentrations and preserved at 17ºC

		ATP Content (pmol/10^6 spermatozoa)		
		Day 1	**Day 4**	**Day 7**
17ºC (Unstimulated)	**BTS**	119.0±21.4^{a,1}	138.0±13.4^{a,1*}	107.2±13.1^{a,1}
	RSV33	22.9±5.0^{a,2}	49.3±13.3^{a,2*}	34.0±5.0^{a,2}
	RSV100	8.2±1.9^{a,2}	8.7±2.3^{a,3}	9.5±2.7^{a,2}
40 min, 38.5ºC, 5% CO_2 (Stimulated)	**BTS**	104.6±17.2^{a,1}	94.4±18.6^{a,1*}	98.7±17.0^{a,1}
	RSV33	20.8±3.8^{a,2}	19.8±3.8^{a,2*}	26.2±5.1^{a,2}
	RSV100	6.9±1.8^{a,2}	7.3±2.2^{a,2}	4.5±2.1^{a,2}

Boar seminal doses were preserved at 17ºC during 7 days in BTS in absence or presence of different concentrations of RSV (33 and 100 µM). Spermatozoa ATP content was measured as described in Materials and Methods. Results are expressed as mean ± standard error of the mean (SEM) (n=6). Within a RSV concentration (row), different superscripts (^{a,b,c}) indicate statistical differences between days. Within a preservation day (column) differences between RSV concentrations are indicated by different numerical superscript (^{1,2}). Within a day of preservation and treatment, differences between unstimulated and stimulated are indicated as (*).

4. Discussion

At present, a large number of studies show that the natural polyphenol resveratrol may be beneficial to many aspects of cell function and animal health; however, this compound displays a dichotomy: low doses can improve cell function while high doses increase cell death with a concomitant decrease in the mitochondrial membrane function (Fulda et al., 2010; Low et al., 2010; Pervaiz & Holme, 2009; Sareen et al., 2006; Zini et al., 1999; Zunino & Storms, 2006). Regarding male reproductive system, some studies have focused in the actions of resveratrol with divergent results (Branco et al., 2010; Collodel et al., 2011; Garcez et al., 2010; Juan et al., 2005; Ourique et al., 2013; Silva et al., 2012).

In the present study, results obtained with conventional parameters to determine the quality of boar semen doses do not allow to respond to the question of whether RSV addition improves preservation of semen doses stored at 17ºC or whether has an adverse effect. 1) Our data do not show any adverse effect of RSV on the plasma membrane integrity (viability) of boar spermatozoa preserved in seminal doses in BTS at 17ºC with no cytotoxic activity at any dose or time evaluated; thus boar spermatozoa are less sensitive to the possible harmful of RSV than swim-up selected human spermatozoa where treatment with 100 µM RSV caused almost a 100% of spermatozoa death (Collodel et al., 2011); 2) Moreover, our study shows that supplementation of boar semen with high doses of RSV causes a small decrease in the percentage of total motile spermatozoa as well as in spermatozoa velocities after 4 and 7 days storage whereas low doses of RSV are without any effect on sperm motility parameters. Similar results were obtained in swim-up selected human spermatozoa where high doses of RSV were able to inhibit progressive motility whereas low doses of RSV cause high values of progressive motility (Collodel et al., 2011); however, the addition of RSV before ram sperm cryopreservation was without effect on progressive motility (Silva et al., 2012), whereas addition of RSV to ram spermatozoa kept at 5ºC for 9 days induced a significant lower reduction in spermatozoa motility (Sarlos et al., 2002); 3) Regarding RSV effect in the plasma membrane phospholipid disorder and acrosomal membrane integrity during boar semen storage, results obtained are similar to those in sperm motility. Whereas high doses of RSV show a slight negative effect in the percentage of spermatozoa showing high plasma membrane phospholipid disorder after 7 days liquid storage, however, RSV doses of 10-66 µM µM are without any effect in these parameters. In the case of ram semen cryopreserved with RSV there were no differences among different doses (from 5 to 20 µg/mL) with regards both plasma membrane or acrosome integrity (Silva et al., 2012) or even a decrease was observed in the percentage of ram spermatozoa with acrosomal damage after long term storage at 5ºC (Sarlos et al., 2002). In summary, the use of conventional sperm parameters to test the quality of boar semen doses incubated in presence of RSV does not allow us to point to a potential beneficial effect of this polyphenol in the cellular function of spermatozoa during boar semen storage at 17ºC.

Contradictory results with RSV supplementation could be attributed to RSV action being affected by several factors including animal species under study, extender medium used or to the treatment of sperm samples (cryopreservation, swim-up or refrigerated storage). Moreover, conventional sperm parameters used to test the quality of boar semen doses mostly are too insensitive to assess the fertilization potential of liquid stored semen (Waberski et al., 2011). In recent studies, effort has been focused to test whether spermatozoa membranes retain their ability to respond to oviductal signals, considering dynamic responses in spermatozoa under experimentally mimicked fertilizing conditions (Henning et al., 2012; Petrunkina et al., 2005a). Several studies have indicated that a certain subpopulation of boar spermatozoa may loose their responsiveness to the capacitating stimulus bicarbonate during hypothermic storage (Green & Watson, 2001; Guthrie & Welch, 2005; Harrison et al., 1996; Petrunkina et al., 2005b). Henning et al. (2012) showed that the comparison of calcium-dependent spermatozoa responses between three different media, capacitating Tyrode's medium, (including calcium and bicarbonate), bicarbonate-free Tyrode's medium and calcium- and bicarbonate-free medium, sensitively detected storage-related changes in the spermatozoa population. Our results show that previous incubation in the presence of RSV 33 µM is unable to revert the decline in the response to bicarbonate and therefore the increasing inherent instability brought about by liquid storage time of boar semen. Moreover, conservation of seminal doses in the presence of RSV 100 µM induces a significant higher reduction in the response to bicarbonate together with a significant increase in the response to calcium in extended boar semen stored at 17ºC. As calcium is a source of cell instability this loss in the calcium homeostasis is proposed to be the best parameter to assess the destabilization of spermatozoa membrane (Henning et al., 2012). Our results show that addition of RSV to extended boar semen stored at 17ºC induces a higher loss of calcium homeostasis related with a greater membrane destabilization which compromises capacitation dynamics and thereby the chances of fertilization.

Our more evident result is likely the diminution of mitochondrial membrane potential observed in spermatozoa treated with any concentration of RSV (10-100 µM). Similar results were obtained in frozen-thawed ram spermatozoa showing the control group a higher proportion of spermatozoa with high mitochondrial membrane potential than in RSV 20 µg/mL treated group (Silva et al., 2012). The decrease in the mitochondrial membrane potential caused by RSV has been widely described in somatic cells and has allowed its use in cancer treatment since RSV is able i) to regulate the mitochondrial permeability transition pore (Zunino & Storms, 2006), ii) to increase the mitochondrial superoxide production (Low et al., 2010), iii) to decrease the mitochondrial membrane potential (Sareen et al., 2006; van Ginkel et al., 2007) and iv) to disrupt the mitochondrial respiratory chain (Zheng & Ramirez, 2000; Zini et al., 1999). The ATP content diminution in RSV treated boar spermatozoa is very clear in our study, falling between 3-6 times when using 33 µM RSV and 10 to 20 times when using 100 µM RSV. Spermatozoa movement requires ATP hydrolysis catalysed by dynein ATPases localized at the axoneme (Tash 1989). The amount of ATP in non-treated samples does not vary along the time of preservation ($p>0.05$), which is in agreement with previous results from Long and Guthrie (2006) in boar seminal doses, although our values are slightly higher. We have obtained much lower values of ATP in spermatozoa treated with 33 and 100 µM RSV compared with control spermatozoa at any time of preservation, which can be the cause for a parallel decrease in sperm motility. Positive correlations were found between the amount of ATP and the percentage of motile spermatozoa in the RSV treated samples compared to control. This finding confirms the work of Gogol et al. (2009) demonstrating that when ATP levels of boar seminal doses decreases, the percentage of motile spermatozoa also decrease. In addition, we have found a significant correlation between spermatozoa ATP level and velocities that may explain why those spermatozoa from seminal doses treated with RSV that contain lower amount of ATP than control (BTS), move with lower velocities.

5. Conclusions

Besides its possible negative effect in spermatozoa function, addition of RSV has been recommended in human sperm prior ICSI because it reduces sperm DNA damage during the process of cryopreservation (Branco et al., 2010), prevents lipid damage induced by cryopreservation (Garcez et al., 2010) and is able to protect sperm against oxidative stress (Collodel et al., 2011). However, in boar spermatozoa, the loss in specific reactivity to capacitating stimuli together with the fall in ATP content, the mitochondrial membrane potential reduction, the increase of plasma membrane phospholipid disorder and the lower percentage of motile spermatozoa lead us to assume that RSV treated samples could be considered subfertile compared with semen stored hypothermically in the standard extender BTS. Our results do not support the use of RSV as a potential supplement to improve the quality of extended boar semen during storage at 17ºC.

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Estimation of Technical, Scale and Economic Efficiency of Paddy Farms: A Data Envelopment Analysis Approach

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Abstract

The present study attempted to analyse the efficiency level of paddy farms in Madurai district of Tamil Nadu state in India. The input oriented Data Envelopment Analysis (DEA) was employed to estimate technical, pure technical, scale, allocative and economic efficiency in the selected paddy farms. The results of the study showed that there exist potential for increasing the paddy output further by 20 percent in the farm holdings by following the best-practices of efficient farms. The study also revealed that 36 percent of the farm were operating at optimal scale and more than seventy per cent of the farms were operating below 50 percent of allocative and economic efficiency levels. The findings also indicated that all the farm inputs were used excessively by the sample farms; the excessive use of nitrogen and women labour was found in the case of fifty percent of the sample farms.

Keywords: technical efficiency, pure technical efficiency, scale efficiency, allocative efficiency, economic efficiency, data envelopment analysis

1. Introduction

Paddy is an important cereal crop consumed by most of the people across the globe and its cultivation provides livelihood security for more than two billion people. Paddy is the major food grain consumed in most of the Indian states and plays a major role in Indian economy. The area under paddy has increased from 31.29 million hectares in 1953-54 to 42.56 million hectares in 2010-11. Similarly, the paddy productivity increased from 902 kg/ha to 2240 kg/ha during the same period. As per 2010-11 statistics, paddy accounted for 33.85 per cent of the total area under food crops and also constituting 42.79 per cent of total area under cereal crops.

However, in the last 20 years, there was sluggishness in further increment of yield and the expansion of area under paddy in India. This sluggishness in the production of paddy would be due to inadequate water for irrigation and the increasing cost of farm inputs. Inter-regional fluctuations in production and productivity of paddy was observed with stagnating yields and ever increasing input costs across India. This increasing cost of inputs would create a situation where farmers may be disinterested to continue paddy cultivation as a profitable enterprise.

It is estimated that India will have to produce more paddy to meet the growing demand of 130 million tonnes of milled rice in the year 2030. Deshpande and Bhende (2003) stated that the productivity increment is the only way out to fill the existing gap between demand and supply of food grain production, as the scope for further expansion of the area is very limited to meet out the requirement of ever increasing population in future. The productivity can be increased through introducing new technologies, adoption of existing technologies and efficient use of available resources. But, introducing new technologies is meaningless unless the existing technologies are used to their full potential (Kalirajan et al., 1996). Available literature indicates that farmers in the developing countries fail to exploit the full potential of a technology and also make allocative errors (Kalirajan & Shand, 1989; Bravo-Ureta & Evenson, 1994; Shanmugan & Palanisami, 1994; Sharma & Datta, 1997). Thus, increasing the efficiency in production assumes greater significance in attaining potential output of the farms. Further, the examination of existing gap between the potential and actual yields on the farm, given the technology and resource endowment of farmers, would provide a better understanding of the yield gap along with the causative factors. Thus, technical efficiency (TE) is an indicator of productivity differences across farms.

It may help in exploring the potentiality of the existing technology. Therefore, enhancing the technical efficiency at farm level is the key to meet the requirement of food grains for the growing population in near future.

The yield gap of paddy is 40 per cent, even though many technologies are available currently to raise the yield of rice in India. In order to minimize the yield gap of paddy, the technological, infrastructural, social and policy-related constraints have to be removed. With this end in view, the present study focuses on measuring the efficiency of paddy production at farm level in Madurai district of Tamil Nadu state in India. Apart, the difference in efficiency across farms will be explained, which, in turn, may help the policymakers in identifying appropriate policies and strategies to improve efficiency of paddy production.

According to 2008-09 statistics, Tamil Nadu state accounts for 4.24 per cent of the total area under paddy in the country and 5.17 per cent of total production. The area under paddy in Tamil Nadu is almost stagnating over the years while the production and productivity of paddy is exhibiting significant variation over the years along with inter district fluctuations. The total paddy area in Triennium Ending (TE) 2009-10 is 18.55 lakh hectares. The season wise distribution of area under paddy pertaining to kuruvai, samba and navari seasons in 2009-10 indicated that kuruvai paddy (April-July) accounted for 16.61 per cent (3.08 lakh ha) and samba paddy (Aug.-Nov) accounted for 76.54 per cent (14.20 lakh ha) and navarai paddy (Dec.-March) accounted for 6.84 per cent (1.27 lakh ha).

Paddy productivity in Tamil Nadu has always been the above the national average. For instance, the average yield of paddy in the state was 2510 kg/ha during 2008-09 which was 324 kg/ha higher than the national average of 2186 kg/ha. The average productivity of paddy ranged from 2308 kg per ha to 3541 kg/ha in the last decade.

2. Materials and Methods

2.1 Study Area and Data

Madurai district was selected as the universe of the study, since the district is covered under Periyar-Vaigai irrigation system and it is one of the prominent district of Tamil Nadu, where in, paddy is extensively cultivated. A three stage random sampling method was adopted to select the sample respondents. At the first stage, all the blocks in Madurai districts were arranged in the ascending order based on the area cultivated under paddy for the year 2010-11 and three blocks namely Vadipatti, Alanganallur and Madurai East blocks were selected at random. Using the same criterion, three revenue villages from each of the selected blocks were selected at random, constituting nine revenue villages at second stage. At the third stage, using the same criterion, 10 farmers were selected from each of the selected nine revenue villages at random, thus constituting a total sample size of 90 farmers. The primary data on yield, input use pattern, cost of cultivation for paddy pertaining to the agricultural year 2010-11 were collected by personal interview method, using a pre -tested interview schedule along with the secondary information about the study region.

2.2 Analytical Framework

Technical efficiency is the ratio of output to input and it stands for the ability of a farm to produce maximal output from the given resources available in the farm. There are two approaches, which are used commonly, to estimate the technical efficiency. According to Farrell (1957), these approaches are classified into two basic groups: parametric and non-parametric frontier models. Stochastic frontier production function is a parametric method which needs specification of a functional and distribution forms for its joint error structure (Coelli & Battese, 1996). Also, it allows the test of hypothesis concerning the goodness of fit of the model. Data Envelopment Analysis (DEA) is a non-parametric model. It does not necessitate assumptions about the production function and the error term distribution, and therefore potential misspecifications are avoided. Both models have their own demerits. Stochastic frontier model requires specification of technology, which may be restrictive in most cases and estimation of parameters and testing of hypothesis are not possible in DEA model.

2.2.1 Model Specification

In present study, Data Envelopment Analysis model was used to estimate the technical, scale and economic efficiencies. DEA uses linear programming to construct the efficient frontier with the best performing observations of the sample used, so that the frontier envelops all observations (Charnes et al., 1978). The distance from a farm to the frontier provides a measure of its efficiency. DEA also enables to assess under which returns to scale each farm operates and to calculate their scale inefficiency. Calculating efficiency under the assumption of constant returns to scale (CRS) gives the '*overall technical efficiency'* score, while assuming variable returns to scale (VRS) allows calculating one component of this total efficiency score, namely the '*pure technical efficiency'*. The latter captures the management practices, while the residual between total technical efficiency and pure technical efficiency shows whether the farm operates under optimal farm size. This residual is called '*scale efficiency'*. Estimated efficiency scores are ranging from 0 to 1. This means that a farm is operating under fully efficient condition when the efficiency score is one. Thus, the input oriented DEA (minimizing input use to obtain a particular output level) was used to estimate both constant returns to scale (CRS) and variable returns to scale (VRS) models. Under the assumption of constant returns to scale, the following input oriented linear programming model was used to measure the overall technical efficiency of paddy farms (Coelli et al., 1998):

$\mathrm{Min}_{\theta,\lambda}\ \theta$

Subject to

$$-y+Y\lambda \geq 0$$
$$\theta x_i - X\lambda \geq 0$$
$$\lambda \geq 0 \quad (1)$$

where,

y_i is a $m \times 1$ vector matrix of output for i^{th} farm,

x_i is a $k \times 1$ vector matrix of inputs for i^{th} farm,

Y is a $n \times m$ output matrix for 'n' number of farms,

X is a $n \times k$ input matrix for 'n' number of farms,

θ is an efficiency score, it is a scalar whose value would be the efficiency measure for each 'i' farm and it ranges from 0 to 1. If $\theta = 1$, then the farm would be efficient; otherwise, the farm would be below the efficient level, and

λ is a $n \times 1$ vector of matrix which provides the optimum solution. The λ values are used as weights in the linear combination of other efficient farms for an inefficient farm, which influences the projection of the inefficient farms on the calculated frontier.

This CRS is applicable only when all the firms are operating under the optimum scale. But, all firm are not able to operate under optimum condition due to imperfect competition and constraints on finance, etc. So, the estimation under CRS model will results in measure of technical efficiency which are confounded by scale efficiency. The use of the VRS specification will permit the calculation of technical efficiency devoid these scale effects. Thus, the VRS model to measure the pure technical efficiency is specified as the following linear programming model (Banker, Charns, & Cooper, 1984):

$\mathrm{Min}_{\theta,\lambda}\ \theta$

Subject to

$$-y+Y\lambda \geq 0$$
$$\theta x_i - X\lambda \geq 0$$
$$\lambda \geq 0$$
$$N_1 \lambda = 1 \quad (2)$$

where,

N_1 is a $n \times 1$ vector matrix of ones.

In addition, the technical efficiency obtained from the CRS model can be decomposed into two components, one is due to scale inefficiency and one due to pure technical inefficiency. Then difference between the efficiency score estimated from the CRS and VRS gives the scale inefficiency, indicating that return to scale can be increasing or decreasing (Färe & Grosskopf, 1994). The scale efficiency of individual farm was estimated by working out the ratio between technical efficiency scores of CRS and VRS models by following procedure mentioned below:

$$\theta_s = \theta_{CRS}\,(X_K, Y_K) \,/\, \theta_{VRS}\,(X_K, Y) \quad (3)$$

where,

$\theta_{CRS}\,(X_K, Y_K)$ = Technical efficiency under CRS model,

$\theta_{VRS}\,(X_K, Y_K)$ = Technical efficiency under VRS model and

θ_s = Scale efficiency.

It is essential to note that model which is specified for VRS in Equation (2) does not indicate whether the firm is falling in the increasing or decreasing returns to scale region (Coelli et al., 1998). The scale efficiency in Equation (3) is equal to one and confirms that the farm is operating under constant returns to scale. However, increasing or decreasing returns may occur when θ_s is less than one. So, in order to understand the nature of scale inefficiency, another problem of linear programming is necessary to replace the convexity constraint $N_1\lambda = 1$ in model (2) with $N_1\lambda \leq 1$ and $N_1\lambda \geq 1$ for the models of non-increasing returns and non-decreasing returns, respectively. Thus, the following models could also be employed for the measurement of the nature of efficiency.

Non-increasing returns:

$\text{Min}_{\theta,\lambda}\ \theta$

Subject to

$$
\begin{aligned}
-y+Y\lambda &\geq 0\\
\theta x_i - X\lambda &\geq 0\\
\lambda &\geq 0\\
N_1\lambda &\leq 1
\end{aligned}
\tag{4}
$$

Non-decreasing returns:

$\text{Min}_{\theta,\lambda}\ \theta$

Subject to

$$
\begin{aligned}
-y+Y\lambda &\geq 0\\
-y+Y\lambda &\geq 0\\
-y+Y\lambda &\geq 0\\
\theta x_i - X\lambda &\geq 0\\
-y+Y\lambda &\geq 0\\
\lambda &\geq 0\\
N_1\lambda &\geq 1
\end{aligned}
\tag{5}
$$

Besides these efficiency measures, allocative efficiency (AE) and economic efficiency (EE) were estimated to measure the farms' ability to allocate farm inputs optimally with their given respective input prices. The following linear programming model was used to estimate the economic efficiency of the firms:

$\text{Min},_{\lambda,x_i*}\ w_i'\ x_i*$

Subject to

$$
\begin{aligned}
-yi + Y\lambda &\geq 0,\\
x_i* - X\lambda &\geq 0\\
N_1'\lambda &= 1\\
\lambda &\geq 0,
\end{aligned}
\tag{6}
$$

where, w_i is vector of input price of i^{th} firm and x_i* (which is calculated by LP) is the cost-minimizing vector of input bundles of i^{th} farm, given the input price w_i and the output levels y_i. The economic efficiency for firm 'i' was then solved by the following computation:

$$EE = w_i'x* \ / \ w_i'\ x_i \tag{7}$$

That is, the observed cost is compared to the minimum cost which the firm would face, if using the optimal input

$$AE = EE \ / \ TE \tag{8}$$

Which measures firm i's relative ability to allocate the input-bundle in the cost-minimizing way, given the estimated technology. Also, this procedure includes any slacks into the allocative efficiency measure. This is often justified on the grounds that slack reflects an inappropriate input mix (Ferrier & Lovell, 1990).

All the models given above were solved for each individual sample farms. Paddy production (Y) in kg was used as output and men labour in man days, total women labour in women days, seeds in kg, plant nutrients (Nitrogen, Phosphorus, Potash) in kg and machine labour in hours were used as inputs (X). DEAP version 2.1 was used to solve all the above said input oriented DEA models.

3. Results and Discussion

3.1. Summary Statistics of Sample Farms

The summary statistics of paddy sample farms is presented in Table 1. It is observed that the significant difference exist among the farmers in the input usage and output realised. The paddy farm size ranged from 0.14 ha to 10 ha with the mean size of 2.11 ha. The yield of paddy realised by in sample farms ranged from 1616.01 kg/ ha to 6200.96 per/ha with the mean yield of 4456.40 kg/ha. The per ha mean usage inputs varied widely and in the case of seed it was 82.88 kg; similarly for labour it was 28.73 days of men labour and 135 days of women labour. Regarding fertiliser and machine power, the per hectare usage stood at 102.23 kg of nitrogen, 63.32 kg of phosphorus, 73.49 kg of potash and 16.13 machine hours.

Table 1. Summary statistics of sample farms

Inputs	Mean value	Minimum Value	Maximum Value
Farm Size in ha	2.11	0.14	10.0
Yield in Kg/ha	4456.40	1616.01	6200.96
Seed in Kg/ha	82.88	50.0	125.0
Men labour in days/ha	28.73	8.23	60.10
Women labour in days/ha	135.17	37.05	193.26
Nitrogen in kg/ha	102.23	48.16	227.24
Phosphorus in kg/ha	63.32	28.40	113.62
Potash in kg/ha	73.49	37.05	222.3
Machine labour in hours/ha	16.17	2.06	27.79

3.2 Overall Technical Efficiency, Pure Technical Efficiency and Scale Efficiency

The results of the DEA of paddy farms in Madurai district are given in Table 1. About 30 per cent of the sample farms were falling under the efficiency group (100 per cent) with the assumption of constant return to scale (CRS), whereas sample farms falling under least efficiency group (below 50 per cent) constitutes only 2.22 per cent of sample farms. This finding indicated that the most of the farms in the study area were not technically efficient with respect to input usage in paddy production. Moreover, the overall technical efficiency of sample farms was ranging from 0.42 to 1.00 with mean efficiency score of 0.80. Similarly, the pure technical efficiency score was ranging from 0.44 to 1.00 with mean efficiency score of 0.85 and scale efficiency score was ranging from 0.62 to 1.00 with mean efficiency score of 0.95. Thus, the mean level of overall technical inefficiency was estimated as 20 per cent for paddy farms in the study region. This result succinctly brought out the fact that the farmers were not utilizing their production resources efficiently and also indicating that they were not obtaining maximal output from the given level of inputs available with them. In other words, technical efficiency among the sample farms can be increased by 20 per cent through adoption of best practices of efficient farms. The decomposition of overall technical efficiency is given in Figure 1. As shown in the figure, pure technical inefficiency accounts for 15 per cent and scale inefficiency accounts for 5 per cent in overall technical efficiency of the paddy farms.

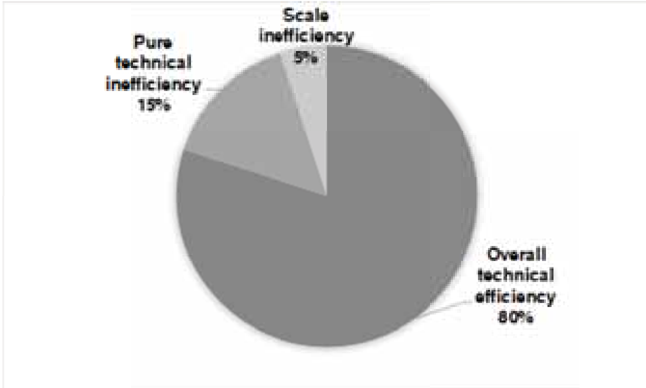

Figure 1. Percentage of pure and scale inefficiency in overall technical efficiency

Table 2. Frequency distribution and summary statistics on overall technical efficiency, pure technical efficiency, scale efficiency, allocative efficiency and economic efficiency measures in sample paddy farms

Efficiency level	Overall technical efficiency		Pure technical efficiency		Scale Efficiency		Allocative efficiency		Economic Efficiency	
	No. of farms	**Per cent**	**No. of farms**	**Per cent**	**No. of farms**	**Per cent**	**No. of farms**	**Per cent**	**No. of farms**	**Per cent**
Below 50	2	2.22	2	2.22	0	0.00	67	74.44	78	86.67
50-60	13	14.44	6	6.67	0	0.00	13	14.44	5	5.56
60-70	14	15.56	12	13.33	1	1.11	3	3.33	0	0.00
70-80	14	15.56	11	12.22	6	6.67	2	2.22	2	2.22
80-90	12	13.33	13	14.44	12	13.33	3	3.33	3	3.33
90-100	8	8.89	12	13.33	38	42.22	1	1.11	1	1.11
100	27	30.00	34	37.78	33	36.67	1	1.11	1	1.11
Total No. farmers	90	100.00	90	100.00	90	100	90	100.00	90	100.00
Minimum	0.42		0.44		0.62		0.22		0.18	
Maximum	1		1		1		1		1	
Mean	0.80		0.85		0.95		0.46		0.38	
Median	0.80		0.92		0.99		0.45		0.34	
Standard Deviation	0.17		0.16		0.08		0.15		0.17	

3.3 Allocative and Economic Efficiency

With respect to allocative efficiency, about 77.44 percent of sample farms were operating under below 0.50 level of allocative efficiency score. The implication of this result is that majority of the respondents are not allocatively efficient in the use of production resources. Furthermore, allocative efficiency among the respondents was ranging between 0.22 and 1.00, with a mean allocative efficiency score of 0.46. This result revealed that 54 percent of resources are inefficiently allocated relative to the best-practiced farms producing the same output and facing the same technology in the study area. This advocated that allocative efficiency among the respondents could be increased by 54 per cent in the study area through the better utilization of resources in optimal proportions for respective paddy production with the current state of technology. This would enable the farmers to equate the marginal revenue product (MRP) of input to the marginal input cost, thereby improving farm income.

As regards the economic efficiency, majority of the respondents (86.67 percent) operated within an efficiency score level of less than 0.50. This implied that majority of the respondents were not economically efficient in the use of production resources. This can result in higher costs per unit of output for a farm firm and hence the inability of the farmer to maximize profit. Moreover, economic efficiency score among the respondents varied widely ranging between 0.18 and 1.0, with a mean economic efficiency score of 0.38. This result suggested that the farmers in the study area were not able to minimize the cost of production. In other words, about 72 per cent of production costs were wasted relative to the best practiced farms producing the same output and facing the same technology in the study area. This exposed that overall economic efficiency among the respondents could be increased by 72 per cent through reduction in production costs that would occur if production were to occur at the allocatively and technically efficient point given the current state of technology. This would enable the farmers to minimize production costs, and hence maximize income and profit.

3.4 Scale of Operations in the Production Frontier

It important to know about the number of farmers are operating in three regions of production scale such as optimal scale, sub-optimal scale and supra-optimal scale. As shown in the Figure 2, of the total sample farmers, 28.89 per cent farmers are operating under sub-optimal situation, 34.44 per cent farmers are operating supra-optimal scale and 36.67 per cent of the farmers are operating at optimal scale condition. The results of the distribution of scale of operations in the production revealed that the about 29 per cent of the farmers had the scope to increase production by decreasing the input cost and about 34 per cent of famers could increasing their technical efficiency by reducing the production level.

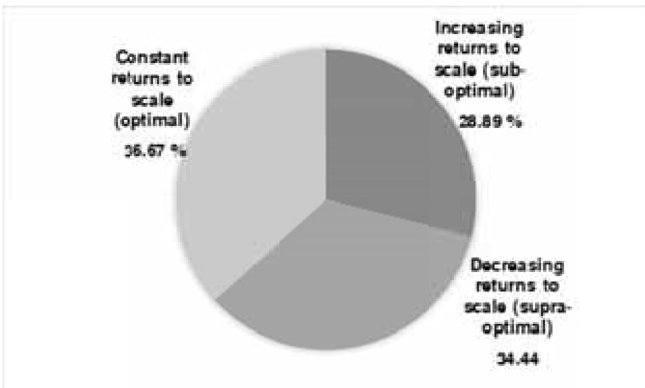

Figure 2. Distribution of scale of operation of paddy farms

3.5 Input Slacks and Number of Farms Using Excess Inputs

The mean input slacks and excess input use percentages are given in Table 2. Since a slack indicates excess of an input, a farm can reduce its expenditure on this input by the amount of slack without reducing its output. The greatest slacks were in women labour use followed by nitrogen fertilizers, seed materials, potassium fertilizers, men labour, machine labour and phosphorus fertilizers use. Out of the total number of farmers, about 51.11 per cent farmers were using excess women labour, followed by nitrogen fertilizers (50.00 per cent), men labour (28.89 per cent), potassium fertilizers (25.56 per cent), seed materials (24.44 per cent), machine labour (18.89 per cent) and phosphorus fertilizers (6.67 per cent).

Table 3. Input slacks and number of farms using excess inputs

Inputs	No. of Farms	% of farmers	Mean input Slack	Mean inputs used	Excess input use in per cent
Seed in Kg/ha	22	24.44	6.44	83.13	7.75
Men labour in days/ha	26	28.89	1.91	32.47	5.87
Women labour in days/ha	46	51.11	15.61	137.42	11.36
Nitrogen in kg/ha	45	50.00	13.45	113.47	11.85
Phosphorus in kg/ha	6	6.67	0.36	68.19	0.53
Potash in kg/ha	23	25.56	5.75	70.69	8.13
Machine labour in hours/ha	17	18.89	0.85	16.21	5.22

4. Conclusion

1) The results of the study showed that the technical efficiency score was 0.80. This indicated that there exist still a potential of 20 percent for increasing the paddy output of the farmers, if the production gap between the average and the best-practice farmers is narrowed.

2) The study showed that more than 60 percent of the farmers have scale efficiency which indicated that majority of farmers are not operating at the optimal scale and these farmers are operating very far away from the efficiency frontier. Also, the overall technical inefficiency among the respondents was attributed more to scale inefficiency than to the pure technical inefficiency.

3) In terms of allocative efficiency, still there is a potential of 54 percent for increasing output through optimal allocation of given inputs. With respect to economic efficiency, the results indicated that 87 percent of farmers are economically inefficient with a mean efficiency score of 0.38. This implied that there exist enormous potential for the farmers to increase output by adopting the best technology practices of optimal farms through optimal resource allocation.

4) Apart from these finding, it is curious to note that all the inputs were used excessively in the study region, particularly in the case of women labour by about fifty one percent of the farmers and in nitrogen by about fifty percent of the farmers.

5) The findings of the study emphasize the need to improve the overall technical and economic efficiency in the paddy growing farms of the region. Immediate steps on the part of government to ensure the provision of the necessary education, training, extension to bring in social change among farmers and other necessary support in the form of credit and other infrastructures in order to bring in technically and allocatively efficient paddy production in the study region.

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Farmers' Perception of and Coping Strategies to Climate Change: Evidence From Six Agro-Ecological Zones of Uganda

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Abstract

In Uganda, weather-related events such as prolonged dry seasons, floods, storms, mudslides, extreme rainfall, and delayed/early rains have become more frequent and/or intense. This has left most of the rural poor farmers' food insecure and their livelihoods threatened. A total of 192 sweetpotato farmer households distributed in six agro-ecological zones were interviewed to assess how farmers perceive the effects of changes in climatic variables, and how they have adjusted their farming practices to cope with the changes in climate. Gender of the household head and size of land owned significantly affected adaptation. Ninety nine percent of all households interviewed had observed a change in the climate in the last 10 years. Drought and floods had the highest impact on crop production across agro-ecological zones. Coping strategies towards extreme events included storing food, income diversification and digging drainage channels. Other strategies were planting trees; high-yielding, early-maturing, drought-tolerant, disease and/or pest-resistant varieties; planting at onset of rains; increased pesticide/fungicide application among others. The smallholder farmer households studied have a high awareness of changes in rainfall and temperature and have taken measures to cope with effects of a changing climate.

Keywords: climate variability, agriculture, farm-level adaptation, smallholder farmers, Uganda

1. Introduction

Global climate change is one of the most critical challenges facing the international community today. Climate change is threatening to undo decades of development efforts due to its negative impacts on agriculture, health, environment, roads, and buildings especially in developing countries (GoU, 2007; IPCC, 2007; Mendelsohn et al., 2006; Stern, 2007). From a food security perspective, sub-Saharan Africa (SSA) is arguably the most vulnerable region to many adverse effects of climate change due to a very high reliance on rainfed agriculture for basic food security and economic growth, and entrenched poverty (Dixon et al., 2001; IPCC, 2007; Cooper et al., 2008). Climate change is certain to amplify these vulnerabilities given projections of warming temperatures, potential for increased activity attributable to the El Niño Southern Oscillation and trends of increased aridity in southern Africa and other regions within Africa (Christensen et al., 2007; IPCC, 2007).

In countries like Senegal, China, Ghana, Nepal, Bangladesh, Nigeria, United States of America, farmers have been mentioned to perceive and even adapt to changes in the climate (Mertz et al., 2009; Byg & Salick, 2009; Fosu-Mensah et al., 2010; Maharjan et al., 2011; Haque et al., 2011; Salau, 2012; Arbuckle et al., 2013). Socioeconomic and environmental factors have been demonstrated in various studies to influence farmers' perception and adaptation to changes in the climate (Deressa et al., 2011), those include education, household size, livestock ownership, agro-ecological zone, farm size and access to credit among others. However, Gukurume (2013) reported that peasant farmers in Bikita district, Zimbabwe, had no adaptive capacity due to extreme poverty levels and reliance on basic technologies.

Coping strategies that have been recommended for instance in Ethiopia to lessen the negative impacts of climate change include encouraging livestock ownership, planting early-maturing and drought-tolerant crop varieties, investment in irrigation, and strengthening research institutions (Deressa & Hassan, 2009). In Ghana, Fosu-Mensah et al. (2010) identified crop diversification and changing planting dates for crop plants as the two most common adaptation strategies used by farmers. In Zimbabwe, crop and livelihood diversification were the main coping strategies used to reduce the risk of crop failure and livelihood vulnerability (Gukurume, 2013).

Despite Uganda being highly vulnerable to rainfall variability and climatic shocks like droughts and floods (MoWE, 2002; GoU, 2007; GoU, 2010; MoWE, 2010), micro-level studies at the farm-level on how rural smallholder farmers perceive these changes are limited. Most studies assessing the potential effects of climate change on African agriculture are regional or national and yet adaptation is place-based and needs the use of place-specific strategies (Fischer et al., 2002; Hassan & Nhemachena, 2008; Kurukulasuriya & Mendelsohn, 2008; Lobell et al., 2008; Seo et al., 2009; Deressa et al., 2011). There is also limited knowledge on whether farmers perceive climate change and how they are responding to the effects of a changing climate. It is also important to note that local perceptions cannot be estimated by models and the need to document how the lives of the local people are affected by the recent changes in climate.

This study therefore examined how rural smallholder farmers in different agro-ecological zones in Uganda perceive the effects of changes in climatic variables, and how they have adjusted their farming practices to cope with the changes in climate. Place-based perceptions and farm-level coping strategies of resource-constrained peasant farmers in Uganda are not documented. Specifically, we identified the major factors and quantified the extent to which these factors influence perceptions of climate change; further, we investigated actual farm-level coping strategies and documented how changes in climate affect crop production in six different agro-ecological zones of Uganda. We hypothesized that perceptions of and adaptation to climate change is highly influenced by the socioeconomic and environmental factors under which farmers live.

2. Materials and Methods

2.1 Survey Design and Study Area

A cross sectional household survey was carried out using a standard structured questionnaire applying both qualitative and quantitative methods of data collection and analysis. The questionnaire assessed demographic characteristics, perceptions of changes in rainfall, temperature and extreme weather events in the last 10 years, how changes in climate have affected crop production in the last 10 years and the changes made by farmers on their farms within the last 10 years because of changes in climate. The questionnaire was pre-tested on five households in Zirobwe, Luwero district and accordingly revised to produce the final questionnaire that was used in the study.

Interviews were conducted in six districts distributed among six agro-ecological zones of Uganda (Table 1). At least two sub-counties were covered in each district. District selection was based on a representation of the different agro-ecological zones of Uganda. Based on the above criteria a multi-stage, purposive sampling method was used to select the districts and the sub-counties. Selection of the villages and parishes to be surveyed was further guided by knowledge of local leaders and ease of accessibility. Respondents were distributed in 180 villages, 59 parishes, 17 sub-counties and six districts. Enumerators were first trained on the study tools and questionnaires prior to the data collection exercise. One respondent per household and per village or a distance of 5-10 km apart were considered for the study. People who were about 30 years of age or more and had lived in that village for the past 10 years or more were only considered in the study to ensure that respondents make meaningful comparisons between the past and the present. Interviews of the selected respondents were conducted in their homes. The number of respondents interviewed per district was 32 and included male, female, elderly and young farmers. A total of 192 farmer households were interviewed individually between August and October 2011.

Table 1. Characteristics of the six agro-ecological zones (AEZ) under study

AEZ (district)	Description of AEZ	Agricultural practices
Eastern Savannah (Soroti)	Rainfall from 800-1500 mm, 1,200-1,340 m asl., generally flat with undulating hills, moderate to good soils.	Rainfed agriculture, consisting of cereals, oil crops and pulses with moderate livestock rearing. Paddy rice grown in drained swamps.
Lake Albert Crescent (Masindi)	Rainfall from 800-1,400 mm, 620-1,585 m asl., generally flat with undulating hills. Soils are good to moderate.	Rainfed mixed farming of maize *Zea mays* L., pulses, root crops, coffee and livestock rearing.
Lake Victoria Crescent (Wakiso)	Rainfall of 1,200-1,450 mm, 1,000-1,800 m asl., hilly and flat areas, some with wetlands and forest. Soils good to moderate.	Mixed cropping of bananas, Robusta coffee *Coffea robusta* Pierre ex A.Froehner, vegetables, maize and moderate dairy farming. Mostly rainfed.
Northern Farming System (Gulu)	Average rainfall 1200 mm, 975-1,520 m asl., generally flat with isolated hills, fairly heavy fertile soils.	Rainfed crop cultivation, consisting of sorghum *Sorghum* sp., pearl millet *Eleusine coracana* Gaertn., cassava, sesame *Sesamum indicum* L. and pulses. Some rearing of cattle and small ruminants.
South Western Highlands (Kabale)	Rainfall >1400 mm, altitude 1,300-3,960 m mountainous areas of Mt. Muhavura with mostly volcanic rich soils.	Rainfed mixed farming involving mostly stall fed cattle, small ruminants, and vegetables, tubercrops such as potato.
Western Range Lands (Kasese)	Rainfall 915-1020 mm, altitude 600-1,524 m, rolling hills with some flat areas, soils are moderate to poor	Cattle rearing is predominant mixed in places with banana *Musa* sp. production

Source: http://www.fao.org/agriculture/seed/cropcalendar/aezones.do?isocode=UGA.

Table 2. Description of model variables of the selection equation for the Heckman probit selection model

1. Dependent variable **Description**	**Farmers who perceived climate change (%)**	**Farmers who did not perceive climate change (%)**
Perception of climate change (dummy: takes the value of 1 if farmer has perceived a change in the last 10 years and 0 otherwise)	99.0	1.0
2. Independent variables		
Description	Mean	S.D.
Level of education of household head (dummy: takes the value of 1 if above A level and 0 otherwise)	0.06	0.2
Age of the household head (years; continuous)	43.3	12.7
Social capital (dummy: 1 if member of a farmers group and otherwise 0)	0.4	0.5
Off-farm income source (dummy: 1 if present otherwise 0)	0.4	0.5
Weather forecast and climate information (dummy: 1 if received otherwise 0)	0.5	0.5
Traditional knowledge of local early warning signs (dummy: 1 if uses local signs to predict seasons and 0 if otherwise)	0.6	0.5
Extension information on sweetpotato crop (dummy: 1 if received otherwise 0)	0.1	0.3
Farming experience (continuous)	22.3	12.6
Elevation (m above sea level; continuous)	1261.6	380.8
AEZ is Northern farming system (dummy: 1 if Gulu otherwise 0)	0.2	0.4
AEZ is South Western Highlands (dummy: 1 if Kabale otherwise 0)	0.2	0.4
AEZ is Western Range Lands (dummy: 1 if Kasese 0 if otherwise)	0.2	0.4
AEZ is Lake albert Crescent (dummy: 1 if Masindi and 0 if otherwise)	0.2	0.4
AEZ is Eastern Savannah (dummy: 1 if Soroti, 0 if otherwise)	0.2	0.4
AEZ is Lake Victoria Crescent (dummy: 1 if Wakiso and 0 if otherwise)	0.2	0.4

2.2 Statistical Analysis

Data was entered using MS Excel and exported to STATA for basic descriptive statistical analyses. Frequencies and means were the major statistical tools that were used to enable the description of farmers' perceptions about changes in climatic variables and plant health as well the coping strategies being practiced to mitigate the effects of a changing climate.

2.3 Empirical Model

As previous authors have found, perception and coping strategies to climate change are influenced by a number of socioeconomic and environmental factors (Nhemachena & Hassan, 2007; Deressa et al., 2009; Nhemachena, 2009; Deressa et al., 2010). We therefore hypothesized that factors which affect perception and the development of strategies to cope with changes in climate will include agroecology, gender, age, household size, land holding, farm labor, ability to pay/hire labor, ownership of livestock, non-farm income source, access to credit, social capital, farming experience, practice of irrigation, use of farm inputs such as fertilizers and pesticides, receiving weather forecast, intercropping on the farm and the use of local signs to predict seasons. A two-step regression model (Heckman selection model) was used for this study because farmers first perceive and then develop strategies to cope with climate change.

2.4 Dependent and Independent Variables

Dependent variables are:

i) whether a farmer has or has not perceived climate change (Table 3);
ii) whether a farmer has or has not developed coping strategies to climate change (Table 4).

Independent variables include agro-ecological zone and altitude, age and education of the head of the household, household size, land holding, farmlabor, ability to pay/hire labor, ownership of livestock, non-farm income source, access to credit, social capital, farming experience, practice of irrigation, receiving weather forecast, intercropping on the farm, and the use of local signs to predict seasons.

Table 3. Description of model variables of the outcome equation for the Heckman probit selection model

1. Dependent variable		
Description	**Farmers who adapted (%)**	**Farmers who did not adapt (%)**
Adaptation to climate change (dummy: takes the value of 1 if farmer has adapted and 0 otherwise)	85.4	14.6
2. Independent variables		
Description	**Mean**	**S.D.**
Level of education of household head (dummy: takes the value of 1 if above A level and 0 otherwise)	0.06	0.2
Household size (continuous)	7.5	4.3
Gender of household head (dummy: 1 if male otherwise 0)	0.7	0.4
Off-farm income source (dummy: 1 if present otherwise 0)	0.4	0.5
Livestock ownership (dummy: 1 if livestock owned otherwise 0)	0.6	0.5
Extension information on sweetpotato crop (dummy: 1 if received otherwise 0)	0.1	0.3
Total land holding (ha; continuous))	19.8	190.3
Credit (dummy: 1 if there is access otherwise 0)	0.8	0.4
Intercropping (dummy: 1 if farmer intercrops and 0 if otherwise)	0.9	0.3
Irrigation (dummy: 1 if farmer irrigates and 0 if otherwise)	0.1	0.3
Number of farm workers (continuous)	4.5	3.6
Ability to hire farm labor (dummy: 1 if hires labor and 0 otherwise)	0.6	0.5

Table 4. Results of the Heckman probit selection model (two-step)

Independent variables	Regression values for adaptation model		Regression values for perception model	
	Coefficients	**P value**	**Coefficients**	**P value**
House hold size	0.0073	0.8620		
Livestock ownership	0.4894	0.0670		
Total land holding	-0.0261	0.0060		
Access to Credit	0.4977	0.0700		
Intercropping	-0.0558	0.8470		
Irrigation	0.1259	0.7640		
Ability to hire farm labor	0.2482	0.3720		
Number of farm workers	-0.0440	0.4450		
Education	0.0759	0.9020	-0.0233	0.4950
Extension information on sweetpotato	-0.0065	0.9890	-0.0044	0.8910
Off-farm income	0.3850	0.1620	0.0294	0.0117
Gender of household head	0.5734	0.0340	1.1592	0.0000
Age			0.0027	0.0030
Social capital			0.2055	0.1910
Climate information			-0.0044	0.8910
Local knowledge of early warning signs			0.0115	0.4910
Farming experience			-0.0016	0.1010
Elevation			0.0008	0.0000
AEZ is Northern farming system			0.0006	0.9820
AEZ is South Western Highlands			-0.7574	0.0000
AEZ is Western Range Lands			0.0844	0.0010
AEZ is Lake albert Crescent			-0.0241	0.3220
AEZ is Eastern Savannah			0.1324	0.0000
AEZ is Lake Victoria Crescent			-0.0779	0.0180
lambda	0.1036	0.0170		
Number of observations	179			
Censored observations	28			
Uncensored observations	151			
Wald chi^2 3643.88, p=0.000				

3. Results

3.1 Farmers' Perceptions about Changes in Climatic Variables

Nearly all the households (99%) interviewed had observed a change in the climate in the last 10 years. The percentage of households reporting that rain came late (47.6%) were nearly equal to those reporting rain to be coming early (47.1%). Forty three percent reported that rain increased in amount and intensity while 36% perceive rain to be extreme (Figure 1). The highest proportion of farmers (39% households) perceived an increase in temperature in the last 10 years, while 27% of the households did not observe any change in temperature (Figure 2). Extreme climatic events like floods, drought/prolonged dry seasons, and storms were reported to have increased in the last 10 years (Figure 3). Perception to climate change was indeed influenced by the agro-ecological zone; farmers in the northern farming system (Gulu), western range lands (Kasese), and eastern savannah (Soroti) perceiving more changes in climate variables than their counterparts in south western highlands (Kabale), Lake Victoria crescent (Wakiso), and Lake Albert crescent (Masindi).

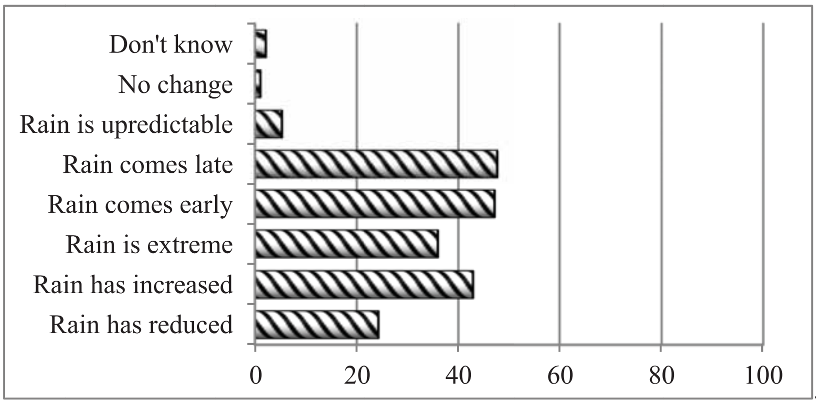

Figure 1. Farmer perception of changes in rainfall in the last 10 years (% households)

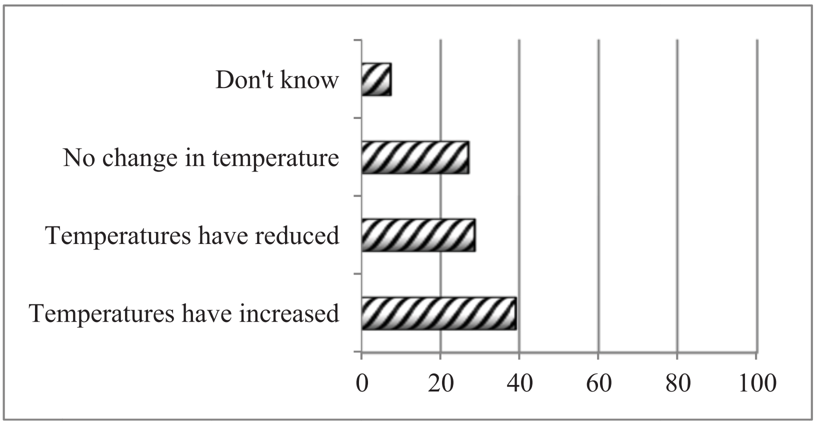

Figure 2. Farmer perception of changes in temperature (% households)

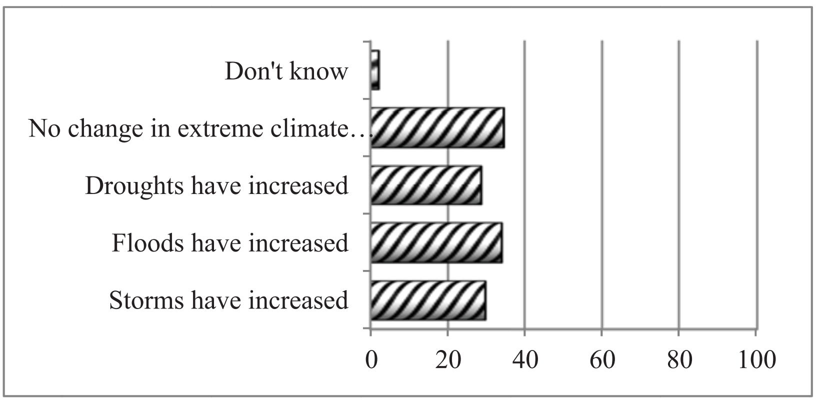

Figure 3. Change in the occurrence of extreme climatic events in the last 10 years (% households)

3.2 Factors Affecting Farmers' Strategies to Cope With Climate Change

Only one variable, gender of the household head, positively and significantly influenced adaptation to climate change (Table 5); i.e., male headed households responded faster developing coping strategies. Other factors that positively affected the development of coping strategies were household size, livestock ownership, access to credit, irrigation practice, ability to hire farm labor at peak seasons, education of household head, and access to an off-farm income source. Larger size of land owned negatively and significantly affected adaptation. Other factors that had a negative relationship to adaptation to climate change included intercropping practice, higher number of farm workers, and access to extension information.

Table 5. How changes in climate affected crop production

Climate change event	Effect on crop production	Crop affected	% households
Floods	rotting of tubers and roots, increased fungal diseases, loss of gardens, reduced yields, soil erosion, premature harvest of grain crops, reduced farm land	sweetpotato, potato, cassava, garden pea *Pisum sativum* L., bean *Phaseolus vulgaris* L., greengram *Vigna radiate* (L.) R. Wilczek, groundnut *Arachis hypogaea* L., tomato, eggplant *Solanum melongena* L., cabbage *Brassica oleracea* L., maize, rice, sorghum, sesame, watermelon *Citrullus lanatus* (Thunb.) Matsum. & Nakai, coffee, Taro *Colocasia esculenta* (L.) Schott , orange *Citrus* spp.	20
Mud slides/land slides	takes away fertile soils, soil erosion, washes away gardens	beans, potato, all crops	1
Drought/ prolonged dry season	complete crop failure, reduced yields, drying up of crops, increased pest damage	sweetpotato, cassava, garden pea, common bean, green gram, groundnut, soya bean *Glycine max* (L.) Merr. , cowpea *Vigna unguiculata* (L.) Walp., sorghum, sesame, maize, millet, red pepper	37
Longer and more rain	longer growing time and increased yields	sweetpotato, cassava, beans, groundnuts, maize	5
Changed onset and cessation of rain season)	poor grain quality at harvest, late planting, reduced yields,	potato, cassava, bean, groundnut, maize, sorghum,	11
Extreme/intensive/ heavy rainfall	rotting of bean pods and tubers, reduced yields, cut-off roads, soil erosion, reduced labor	sweetpotato, potato, bean, groundnut, sorghum	19
Storms (strong winds and/or hailstones)	destroyed leaves, broke shoots and flowers, broke house, reduce leaf quality	sweetpotato, cassava, bean, tomato, maize, sorghum, sesame, banana, pawpaw *Carica papaya* L., tobacco *Nicotiana* spp.	11

3.3 Effect of Climate Change on Crop Production

Changes in climate had already started to impact on farmers' crop production. On average, drought had the highest (37% of the households) impact on crop production followed by floods (20% of the households) across agro-ecological zones (Table 6). For instance, floods caused rotting of potato (*Solanum tuberosum* L.) tubers and sweetpotato (*Ipomoea batatas* (L.) Lam.) and cassava (*Manihot esculenta* Crantz) roots, increased fungal diseases in potato, coffee (*Coffea* spp.) and tomato (*Solanum lycopersicum* L.) crops, reduced crop yields, soil erosion, and premature harvest of grain crops and reduced farm land.

Table 6. Changes made in crop, soil and water management practices in the last 10 years

Change made (adaptation strategy)	Households (%)
Planted trees and/or hedges	45.8
Planted quick-maturing crop variety	35.4
Started or increased use of pesticides or fungicides	33.3
Planted new and/or high-yielding varieties of a crop	26.0
Planted drought-tolerant crop or variety	23.4
Applied mulch in a crop garden	22.9
Planted early or late in the season	19.3
Diversified income source to a non-farm income source	18.2
Planted disease-resistant crop variety	18.2
Practiced soil erosion protection methods	17.7
Stopped planting a crop or variety	12.0
Planted pest-resistant crop or variety	11.5
Practiced zero or no tillage	9.4
Increased irrigation	1.0

Total is more than 100 because of multiple responses, n=192.

3.4 Changes Made with Respect to Crop Varieties and in the Management of Crops, Soils and Water

Farmers were asked for the changes which their households had made over the last 10 years following changes in the climate. Survey findings show that 45.8% of households had planted trees in the last 10 years (Table 7). Fruit trees were mostly planted for commercial sale of fruits and as a source of fuel (charcoal and/or firewood). Trees like pine, *Pinus patula* (Schiede ex Schltdl. & Cham.), and *Eucalyptus* species were planted for their timber, building poles, fuel, and for soil erosion protection on the steep slopes of Kabale. *Pinus* species and *Ficus* species were planted mainly for improving soil fertility due to their association with soil fungus.

3.5 Changes Made in Non-Farm Income Sources

We examined the non-farm activities which farmers had taken on to diversify their income sources and therefore spread the risks associated with farming like total crop failure. A number of activities had been started to increase household cash income and included handcraft making, stone quarrying, retail business, working as a casual worker at another farm and securing salaried employment. No household had any new additional cash income activity in Gulu district (Figure 4). Retail business was the most common type of activity started by households (16%) across the six districts.

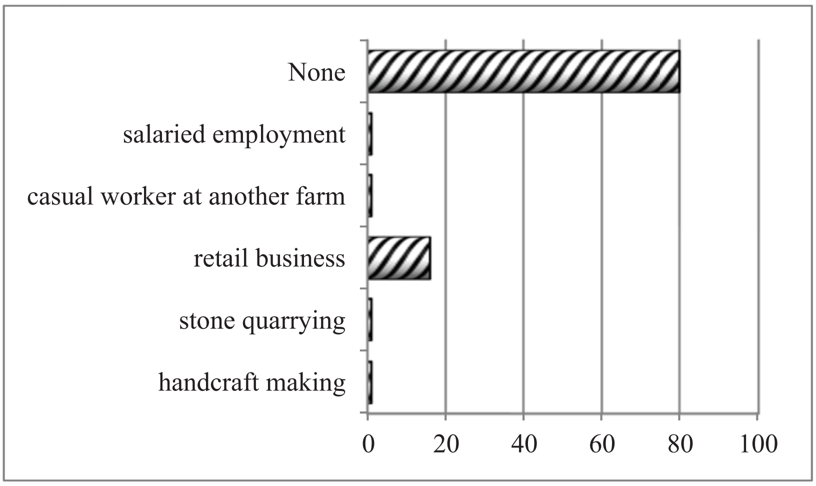

Figure 4. Changes made to diversifying from farm to non-farm activities

4. Discussion

Most of the farmers admitted to observing an increase in the amount and intensity of precipitation and an increase in temperature in the last 10 years. An increase in temperature in the last decades has been recorded in Uganda (MoWE, 2002; Nsubuga et al., 2011) and elsewhere in Africa, e.g., in Ethiopia (Mengistu, 2011), Ghana (Fosu-Mensah et al., 2010), and South Africa (Gbetibouo, 2009). Increased temperatures are said to be responsible for the increased floods reported for instance in Kasese district where the ice cap on Mt. Rwenzori has melted (Masereka & Tenywa, 2000; Kaggwa et al., 2009). In Uganda, higher temperatures have already been reported to reduce crop yields in cereals and coffee reducing the area for coffee cultivation (MoWE, 2002; GoU, 2007; GoU, 2010; MoWE, 2010). Temperature increases have been reported to be responsible for extending the geographic range of some insects (pests and vectors) currently limited by temperature like mosquitoes (*Anopheles* spp.) in highland areas that were historically malaria-free, such as in Kabale, Kisoro and Rukungiri districts of Uganda now also experiencing epidemics (MoFPED, 2009) and the sorghum chafer (*Pachnoda interrupta* Olivier; Coleoptera: Scarabaeidae) which has now extended to the northeastern highlands of Ethiopia including the Afar regions (Demessie, 2004).

Rainfall across the country has been noted to be unreliable and highly variable in terms of its onset, cessation, amount, and distribution, leading to either low crop yields or total crop failure (MoWE, 2002; Mubiru et al., 2012). The increase in rainfall over the years in Uganda agrees with the prediction by the IPCC report of a likely increase in the annual mean rainfall in East Africa (Christensen et al., 2007). If deviations of such magnitude persist a little longer, they could affect the overall physiological activities of plants and result in crop failures and reduced yields. As a result, rainfed agriculture is quite sensitive to even small changes in temperature and rainfall (IPCC, 2007).

Extreme weather events that had been experienced include drought and prolonged dry seasons, floods, storms/extreme rain and mud/landslides in order of decreasing occurrence. It is interesting to point out that extreme weather events have also had positive effects on agriculture like allowing for longer growing times due to plenty of rain hence resulting in increased crop yields especially in the Masindi district, while flooding created new opportunities for growing lowland rice (*Oryza* spp.).

Farmers manage risks, including those related to climate, regularly as part of their everyday lives. However, there is a need for farmers not only to cope with the impacts of a changing climate but rather to adapt in order to reduce the negative impact of climate change. Socioeconomic and environmental factors have a big role to play in the way farmers perceive and later adapt to impacts of a changing climate (Deressa et al., 2011). Coping strategies to protect farmers against climate related hazards included storing food, planting early and digging drainage channels. Other strategies included planting early-maturing varieties, high-yielding varieties, drought-tolerant varieties, disease- and/or pest-resistant varieties, income diversification, tree planting, increased pesticide/fungicide application, among others. Similar coping strategies were reported from various studies conducted in different parts of Africa like Ethiopia, South Africa and Nigeria (Giorgis et al., 2006; Hassan & Nhemachena, 2008; Deressa et al., 2009; Salau, 2012). Adaptive capacity of smallholder farmers to changes in climatic events is usually low due to dependence on natural resources, constraints in human and physical capital, and poor infrastructure (Shewmake, 2008; Salau, 2012; Gukurume, 2013). In this survey, factors that hindered adaptation included poverty (inability to pay for farm inputs, equipment and services like labor), unreliable weather forecasts, and shortage of food to store, among others.

5. Conclusions, Recommendations and Policy Implications

Small-holder farmers need to be supported by government and civil society organizations in the adaptation process to use water resources more efficient in agriculture since rain has becoming more erratic and with delayed onsets of rainfall.

Government institutions need to put more efforts into providing farmers with accurate weather forecasts as most farmers have no confidence in the weather forecasts received. This will enable farmers to fully exploit seasonal rainfall distribution to improve and stabilize crop yields.

There is need for the government of Uganda to facilitate the development and dissemination of agricultural technologies such as integrated pest management (IPM) to substitute the use of pesticides as well as drought-tolerant and early-maturing varieties by research institutions through increased funding to the agricultural sector.

The development and application/use of modeling tools that support adaptation planning and decision making need to be supported and disseminated to scientists and policy makers as this will help in forecasting extreme climatic events like floods, mudslides, disease and pest outbreaks rather than responding to these serious events or outbreaks as is the case in Uganda.

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