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Full Length Research Paper

Determination of method to evaluate parasitism and cover area for studies on *Cotesia flavipes* in sugarcane

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The continual rearing of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) in laboratories (for approximately 30 years) may have led to loss of genetic variability due to drift, selection, and crossing among siblings. This in turn may have compromised the biological characteristics of the insect, notably with respect to its activity level, thereby altering its parasitic and dispersal capacities. This study investigated methods that allowed the testing of parasitism and number of release points per hectare for *C. flavipes*. We tested the efficiency of different colored Moericke traps, yellow Moericke traps containing different concentrations of frass, stick yellow traps arranged at different heights and others containing sugarcane stems, with each stem containing a larva of the sugarcane borer, *Diatraea saccharalis* (Fabricius). Only the use of stems was efficient for measuring cover, aggregation, and geostatistics of its parasitism. The samples showed an aggregated distribution, and the maximum dispersal distance of *C. flavipes* was 25 m. Geostatistical analyses enabled the evaluation and mapping of the number of parasitized larvae. This method permits tests aimed at evaluating quality control during the biological control of *D. saccharalis* with *C. flavipes*.

Key words: Biological control, parasitoid, Hymenoptera, Braconidae, massive release, geostatistic analyses.

INTRODUCTION

Cultivation of sugarcane in large fields creates suitable conditions for pest occurrence and subsequent crop damage. This is particularly applicable to the key pest of sugarcane in Americas - the sugarcane borer, *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) - on account of the frequency of its population surges, high biotic potential, and ability to protect itself from natural enemies (Posey et al., 2001).

The larva of *D. saccharalis* cause direct and indirect damage to sugarcane production. Direct damage is caused by the larvae building galleries in the sugarcane stalks, resulting in weight loss and plant mortality. Indirect damage is due to the invasion of the open galleries by microorganisms, which contaminate the sugarcane juice,

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there by reducing both sugar and ethanol production (Ogunwolu et al., 1991; Dinardo-Miranda et al., 2011).

The use of insecticides to control *D. saccharalis* is highly ineffective after the larva penetrate the sugarcane stalks, thus gaining protection from the insecticidal sprays. The control of *D. saccharalis* is essentially dependent on biological control, mainly by the use of the larval parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) in Brazil (Rossi and Fowler, 2003a and b), Louisiana (Schexnayder Jr. et al., 2001; White et al., 2004; White and Wilson, 2012), Barbados (Alam et al., 1971), and south Texas (Fuchs et al., 1979). This species is native to Indo-Australian region and was introduced into Brazil in 1974 from Trinidad (Botelho, 1992; Kimani-Njogu and Overholt, 1997). Since then, most of the sugarcane fields in Brazil have received mass release of this parasitoid. The total area receiving *C. flavipes* release is 3×10^6 ha (Vacari et al., 2012). Although, biological control is practiced in a large area, there is currently a lack of methods for monitoring the efficacy of parasitoid release.

The success of biological control agents depends on their efficiency to search for and locate target hosts (Nordlund et al., 1988). In spite of the rearing of *C. flavipes* on a large scale since the 1970s, little is known about its quality components, which, according to Boller and Chambers (1977) are related to adaptability, mobility, sexual activity, reproduction, and colonization potential. Some characteristics, particularly flight are very restricted under laboratory conditions (van Lenteren, 1991).

The final steps in biological control, such as the release of natural enemies, are neglected in most of biological control programs, and only a few studies have aimed to determine the best release technique and the adaptations that it may require (Overholt et al., 1994). The continual rearing of *C. flavipes* in laboratories (for approximately 30 years) may have led to loss of genetic variability due to drift, selection, and crossing among siblings (Boller and Chambers, 1977). This in turn may have compromised the biological characteristics of the insect, notably with respect to its activity level, thereby altering its parasitic and dispersal capacities (van Lenteren, 2009).

Most works are carried out using larvae, which infest sugarcane. The disadvantage of using larvae from the fields is in that the distribution of this pest is aggregated. In this context, we aimed to test various methods to study the parasitism and cover area of *C. flavipes* and identify the method that is effective for field studies with this parasitoid in sugarcane field.

MATERIALS AND METHODS

Experiment one - Efficiency of colored Moericke traps to capture *C. flavipes*

The investigation was conducted in Jaboticabal, Sao Paulo, Brazil in a newly planted commercial sugarcane crop of the cultivar CTC-3 in 2009. The plants were 1 month-old (from planting to first harvest, 1.5 years), 0.5 m tall and spaced 1.5 m apart.

In the plot, we designed a concentric circle with a radius of 10 m from the central release point of *C. flavipes*, with an area of 314 m^2. The circumference was divided into four equal parts (resulting in four equal blocks). In each block, we placed equally spaced Moericke traps of different colors trade in the local marked on the edge of the circle (white, blue, yellow, light green, dark green, light blue, dark blue, and light pink randomly distributed in each block) distanced 1.97 m each other and subjected them to eight treatments with four replications. The traps were supplied with 350 ml experimental solution (70 g of salt and 3 ml of detergent per liter of water), and two commercial release receptacles used to trade *C. flavipes* containing together approximately 3,000 adults were released in the center of the circle. The traps were kept in the field for 3 days, and the trap color preference of *C. flavipes* was observed by counting the number of individuals present in the traps.

Experiment two - Efficiency of yellow Moericke traps containing different concentrations of frass

The arrangement, number and distance of traps followed the same pattern as that in "Experiment one", but we added various concentrations of frass of *D. saccharalis* in the solution in the yellow traps since this color are known to attract many species of parasitoids (Hoelmer et al., 1998). We added 1, 2, 4, 6, 8, 10, and 12 g of frass per 350 ml of the solution since frass is a stimuli source that attracts *C. flavipes* (van Leerdam et al., 1985; Potting et al., 1997), and a control without frass. The traps were randomly placed in the area spaced 1.97 m apart each other, consisting of 8 treatments in 4 blocks, and approximately 3,000 parasitoids adults were released in the center of the circle. The traps were kept in the field for three days.

Experiment three - Effect of variation in the height of rectangular yellow sticky traps

The experimental setup was similar to that used in the earlier experiments, but we tested 3 heights for setting the sticky traps (0, 0.25, and 0.50 m) in each block. A randomized complete block design was used, comprised of three trap height treatments in each of four blocks, consisting of 3 treatments in 4 blocks and each trap was a replicate.

Experiment four - Evaluation of *C. flavipes* parasitism by using sugarcane stems containing *D. saccharalis* larva under laboratory conditions

Twenty-seven stems of the cultivar SP80-3280 (susceptible to *D. saccharalis*) were cut using a circular saw. The cut sample comprised one full segment of the sugarcane stem and half of the segments above and below the cut, in order to prevent water loss and ensure continued nutrition and presence of larva inside the stem (Wiedenmann and Smith Jr., 2006). The stems were perforated using an electric drill with a 5/32 mm drill bit, and a single 1.5 cm long *D. saccharalis* larva was inserted into each opening.

Parasitism of *C. flavipes* was evaluated under the following conditions: (one) stems kept vertical, keeping the head of the larva facing upward and the larva on their back; (two) stems in the reversed position; and (three) stems lying prostrate (horizontal). The positions are changed to determine if the methods used to insert the larvae inside the stems or to fix the stems into the soil could compromise the parasitism during the field tests with sentinel caterpillars.

For each position, parasitism was evaluated with either frass lining the opening with frass completely obstructing the opening,

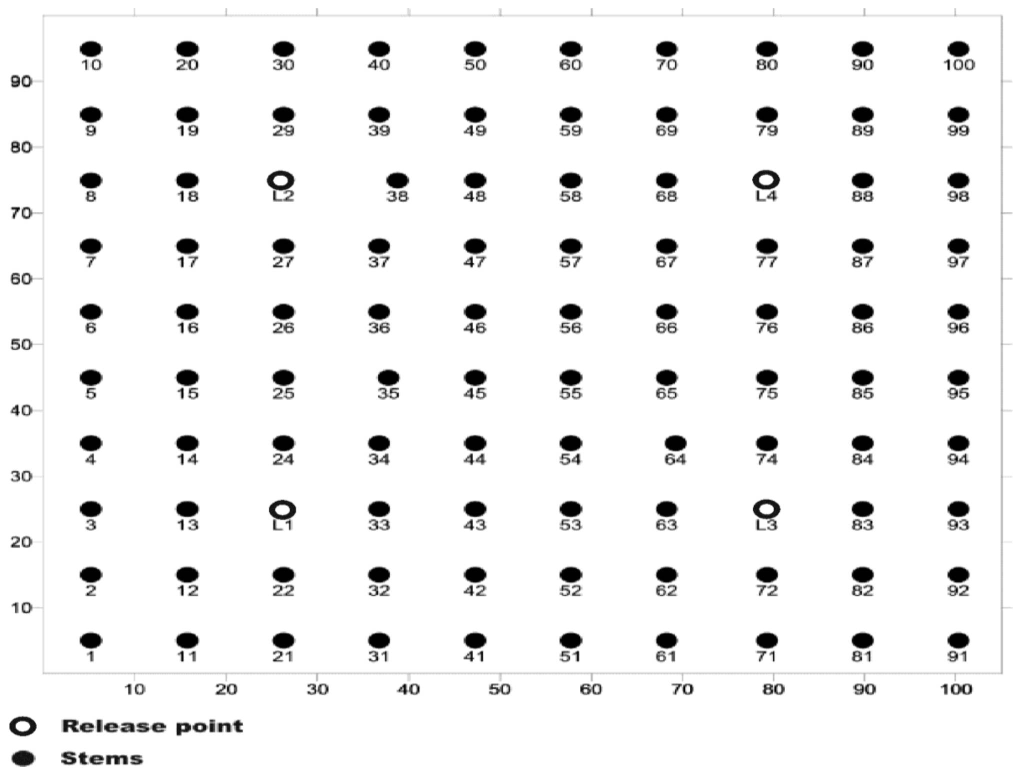

Figure 1. Schematic representation of the sugarcane sample area divided into 100 plots, showing the parasitoid release points and distribution of stems.

And without frass, for a total of nine treatments with three replications.

The stems were placed in a wooden cage (1.0 m × 0.5 m × 0.5 m) covered with the fabric "voile" and exposed to thirty females of *C. flavipes* after eight hours of emergence. After 2 days, the stems were cut longitudinally to retrieve the larvae, which were subsequently maintained in individual 6 cm Petri dishes labeled with the treatment. Each dish contained sufficient artificial diet for the larval development (produced by Sao Martinho mill, Pradopolis, Sao Paulo, Brazil) and was maintained at 25 ± 1°C, 70% ± 10% relative humidity, and a 12 h (L12:D12) photoperiod to evaluate the parasitism.

Experiment five - Determination of spatial distribution of *C. flavipes* by using sugarcane stems containing *D. saccharalis* larvae in field

The experiment was conducted in Jaboticabal, Sao Paulo, Brazil, in a commercial newly planted sugarcane crop of the cultivar CTC-3, covering an area of 10.65 ha. The plants were 7 months old from planting (normal lifecycle, 1.5 years), 2.2 m tall, spaced 1.5 m apart, and moderately resistant to *D. saccharalis*.

The data used to study the spatial distribution of *C. flavipes* parasitism were obtained in an area of 100 m × 105 m, which was divided into 100 plots of 10.5 m × 10.0 m each, considering a border of 10 m from the dirt road. Field sampling of *C. flavipes* populations can be difficult; in previous investigations, attempts to capture the insect by using Moericke traps and yellow adhesive traps have been unsuccessful. It was therefore necessary to count the number of parasitized larvae in each sample unit. The method using stems, which involved the placement of artificially infested sugarcane stems in the field was used as an alternative indicator of *C. flavipes* population distribution and its characteristics. We cut 1,000 stems of the cultivar SP80-3280 (7th harvest, susceptible to *D. saccharalis*), adopting the method described in "Experiment four". The lower segment was cut into a cone shape and inserted into the soil. A single *D. saccharalis* larva was inserted into each opening 1 day before the insertion of the stems into the soil in order to allow sufficient time for the larva to produce frass, because it is known that *C. flavipes* is attracted to the host excrement (Ngi-Song and Overholt, 1997).

In the center of each of the 100 plots, ten sugarcane stems, each containing a single *D. saccharalis* larva was inserted into the soil into the row of the planted sugarcane. The stems were placed in two lines, with five stems in each line and the lines spaced 20 cm apart. Care was taken to insert the stems in such a way that the head of each larva remained facing upward, duplicating the position naturally adopted by the larva.

The parasitoids were released at four points spaced 50 m apart and 25 m from the edge of the main field (Figure 1). One plastic 100 ml receptacle with lid, containing 8 h old parasitoids, was placed at each point. Each receptacle contained approximately 1,500 parasitoids (6,000 insects in total), and the release method was based on Botelho et al. (1980).

The larvae were exposed to *C. flavipes* for 3 days, which is the typical duration of the adult phase of the parasitoid, as has been shown in laboratory tests (Vacari et al., 2012). After this period, the stems were transported in labeled plastic bags [identified with the plot number (1-100)] the laboratory, where they were cut longitudinally to retrieve the larvae and subsequently maintained in individual 6 cm Petri dishes labeled with the plot number. Each dish contained sufficient artificial diet to complete larval development and was maintained at 25 ± 1°C, 70% ± 10% relative humidity, and a 12 h (L12:D12) photoperiod until the parasitoids emerged.

The number of parasitized larvae was counted, together with the number of males, and females that emerged in each plot. The mean, variance, and aggregation indices were determined using the data on parasitized larvae. Subsequently, the data obtained were analyzed using Poisson and negative binomial models. A chi-square adherence test was used to compare the observed and expected frequencies, as described by Anscombe (1949). The indices used to calculate the aggregation and study the spatial distribution of the parasitoids are described as follow:

Aggregation indices

Variance/mean ratio: This index was first used by Clapham (1936) as cited by Perry and Mead (1979). It is also called a dispersion index, and according to Rabinovich (1980), it can be used to measure the deviance of an array of random conditions. Values equal to one indicate a random spatial array; smaller than one, a uniform array; and significantly greater than one, an aggregated array. According to Southwood (1971), the application of this index is affected by the sample unit size and the number of observed individuals. The index is estimated by the following formula:

$I = s^2/m$,

where s^2 is sample variance and m is sample mean.

Morisita's index: This index was developed by Morisita (1959, 1962), with the objective of developing an index independent of the mean sample size and the total number of individuals. Values equal to one indicate a random distribution; more than one, an aggregated distribution; and lesser than one, a uniform distribution. A limitation of the Morisita index is that it may be influenced by sample size (N). Thus, if the index is to be relied upon for accurate data, it is necessary to ensure that the number of sample units is the same in each area being compared. The index is represented by the following formula:

$$I_\delta = N\frac{\sum x^2 - \sum x}{(\sum x)^2 - \sum x},$$

where N is sample size and x_i is the number of insects in the i^{th} sample unit.

The removal of randomness can be tested using the following formula:

$$X_\delta^2 = I_\delta\left(\sum x_i - 1\right) + n - \sum x_i \sim \chi^2_{(n-1)} .$$

If $X_\delta^2 \geq \chi^2_{(n-1 g.l.;0.05)}$, the hypothesis of randomness of distribution should be rejected.

Green's coefficient

Green's coefficient can be used to compare aggregated distributions. Negative values indicate a uniform pattern, whereas positive values indicate an aggregated pattern (Green, 1966). The coefficient is based on the ratio of distribution variance to mean and is expressed by the following formula:

$$C_x = \frac{(s^2/\hat{m}) - 1}{\sum_{i=1}^{n} x_i - 1},$$

where m is sample mean, s^2 is sample variance, and x_i is the number of insects in the i^{th} sample unit.

The k exponent of negative binomial distribution

According to Anscombe (1949), k is estimated for the moment method by equating the first two moments of distribution with the estimated sample moments, resulting in the following expression:

$$k = \frac{m^2}{s^2 - m},$$

where m is sample mean and s^2 is sample variance.

Negative values indicate a uniform distribution. A low positive value (k < 2) indicates a highly aggregated distribution; moderate positive value (k = 2-8), moderate aggregation; and a high positive value (k > 8), a random distribution (Elliott, 1979).

Probabilistic models

Poisson distribution: The Poisson distribution is characterized by variance being equal to the mean ($\sigma^2 = \mu$). The formulas used to calculate the series of probabilities are as follows:

$P(0) = e^{-m}$

$$P(x) = \frac{m}{x} \cdot P(x-1)$$, parameter x = 1, 2, 3,...,

where e is the base of the Napierian logarithm (e = 2.718282...), $P(x)$ is the probability of finding individual x in the sample unit, and m is sample mean.

Negative binomial distribution

The negative binomial distribution is characterized by variance being larger than the mean, indicating aggregated distribution. This index has two parameters: mean (μ) and parameter k (k > 0), and the probabilities are calculated using recurring formulas shown below:

$$P(0) = \left(1 + \frac{m}{k}\right)^{-k}$$, to x = 0

$$P(x) = \frac{k + x - 1}{x} \cdot \frac{m}{m+k} . P(x-1)$$, parameter x = 1, 2, 3,...,

where k is the k exponent of the negative binomial distribution and m is sample mean.

Chi-square goodness of fit test

Chi-square goodness of fit test was used to adjust the data to each probability distribution, using the following expression:

$$X^2 = \sum_{i=1}^{N_c} \frac{(FO_i - FE_i)^2}{FE_i}$$

where N_c is the number of classes of the frequency distribution, FO_i is the observed frequency in the i^{th} class, and FE_i is the expected frequency in the i^{th} class.

The models show a good adjustment to the original data when observed and expected frequencies are reasonably close.

Geoestatistical analyses

The semivariograms were constructed using spherical model for parasitism and Gaussian model for number of males, females and total of emerged adults. Krigeage maps were generated using the data from the semivariograms with the objective of estimating the necessary interpolations to construct isolines and three-dimensional parasitism maps for *C. flavipes.*

Further, geostatistical analyses were conducted using the data on parasitized larvae and the number of males and females emerged in the first generation, in relation to the physical coordinates of the collected samples. Thus, each sample was analyzed in light of the variable value or the number of emerged parasitoids and the coordinates of each point where data were collected, using GPS Garmin Etrex Vista. The Surfer 7.0 software was used to construct a map of the observed values, a semivariogram, and a two-dimensional representation by using isolines and three-dimensional graphics.

RESULTS

Experiment one

C. flavipes was not attracted by the colors of receptacles used in “Experiment one”. However, two males were caught in two light-blue traps (one male in each trap), one male each in light-green and yellow traps, and one female in pink trap. Thus, this test provided evidence that *C. flavipes* is not attracted by color.

Experiment two

C. flavipes was not attracted to the frass dissolved in the solution contained in the traps, showing that the traps used were inefficient in capturing the parasitoids and thus cannot be used in determining spatial distribution.

Experiment three

In this experiment, 60, 25, and four *C. flavipes* adults were captured at the heights of 0, 25, and 50 cm, respectively. The low catch of parasitoids using the traps in experiment one, two and three did not allow performing statistical analyses of data, but showed that Moericke and adhesive traps are not a good tool to catch *C. flavipes.*

Experiment four

D. saccharalis larvae in all the treatments were parasitized, independent of the position of the stem in the cage or the addition of frass in the opening made for the insertion of the larvae.

Experiment five

In this experiment, we recovered 622 of 1,000 *D. saccharalis* larvae from the manually infested sugarcane stems of which 87 were parasitized (13.98% parasitism).

Aggregation indices

We found that the variance values were larger than the mean values for all the evaluated parameters (parasitism, number of females, number of males, and total number of insects). Accordingly, the dispersion index (I) was greater than 1, indicating an aggregated spatial distribution of parasitism, emerged males, emerged females, and total emerged insects (first generation). However, the variance/mean ratio showed that values describing the number of males, number of females, and total number of adults were considerably larger than those describing parasitism. This indicates that aggregation of the first generation of insects was greater than that of the parasitism of the insects initially released in the crop (Table 1).

The values obtained using Morisita’s index (I_δ) were greater than one, confirming aggregated distribution for the evaluated parameters. In fact, X_δ^2 values obtained for the parasitism, number of males, number of females, and total numbers of adults (first generation) confirm higher aggregation for these parameters (Table 1).

The aggregated distribution pattern was further confirmed by calculating Green’s coefficient (C_x). The C_x values obtained were more than zero, which, according to Davis (1993), indicates an aggregated distribution (Table 1).

For the parameter *k*, the estimated number of males, number of females, and total number of emerged adults were slightly more than zero, indicating high levels of aggregation. For parasitism, the *k* value was larger than that obtained for the number of males, females, and total of emerged insects, indicating that parasitism is less aggregated than the number of insects emerged in the first generation (Table 1).

Probabilistic models

Data adjustment for parasitism, number of males, number of females, and total number of adults emerged in the first generation was conducted to study the probabilistic models that explain the spatial distribution of *C. flavipes*, that is, Poisson and negative binomial distributions (Figure 2). The Poisson distribution did not adjust the data for parasitism, number of males, number of females, or total number of insects emerged in the first generation.

Further, the chi-square value was significant with 99% probability, indicating that the distributions of parasitism and number of insects emerged in the first generation were not random. Since the variance was larger than the mean, the data adjustment was tested for negative

Table 1. Means, variances, and dispersion indices for *Cotesia flavipes* in sugarcane crop.

Index	Parasitism	Females	Males	Total insects
m	0.8700	28.7700	12.8300	41.6000
s^2	1.6092	2,289.3304	696.6476	4,972.6869
$I = s^2/m$	1.8496	79.5735	54.2983	119.5357
I_δ	17.7500	3.7047	5.1159	3.8216
X^2_δ	1,824.1385**	7,877.70**	5,375.50**	11,834.00**
d.f.	4	9	28	39
$k_{moments}$	1.0239	0.3662	0.2407	0.3509
C_x	0.0405	0.0273	0.0416	0.0285

m = sample mean, s^2 = sample variance, I = variance/mean ratio, I_δ = Morisita's index, X^2_δ = Chi-square adherence test for the calculated values of Morisita's index, ** = significance level of 1% probability, $k_{moments}$ = k calculated by the moment method, C_x = Green's coefficient.

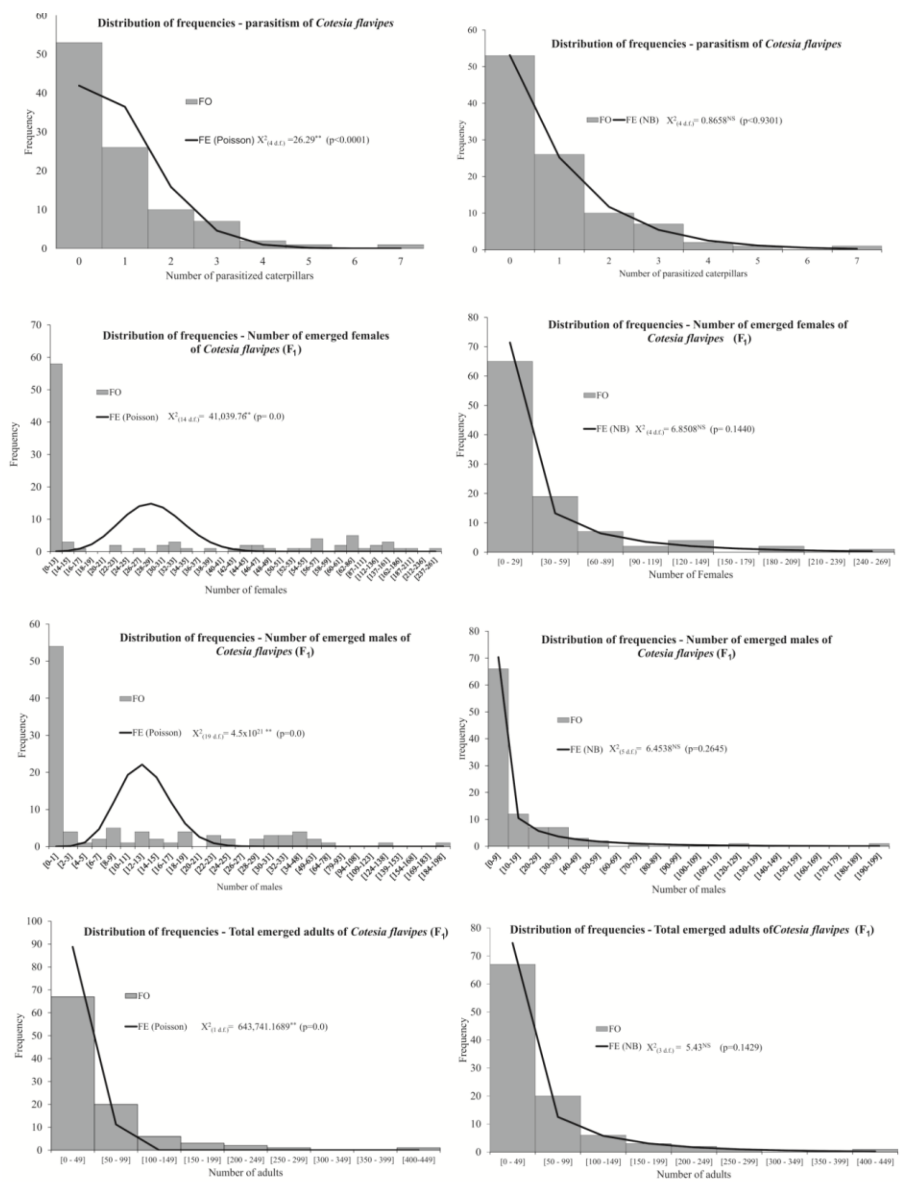

Figure 2. Graphs showing the observed frequencies and data adjustment to Poisson and negative binomial distributions for *C. flavipes* parasitism, number of males, number of females, and total number of adults emerged in the first generation.

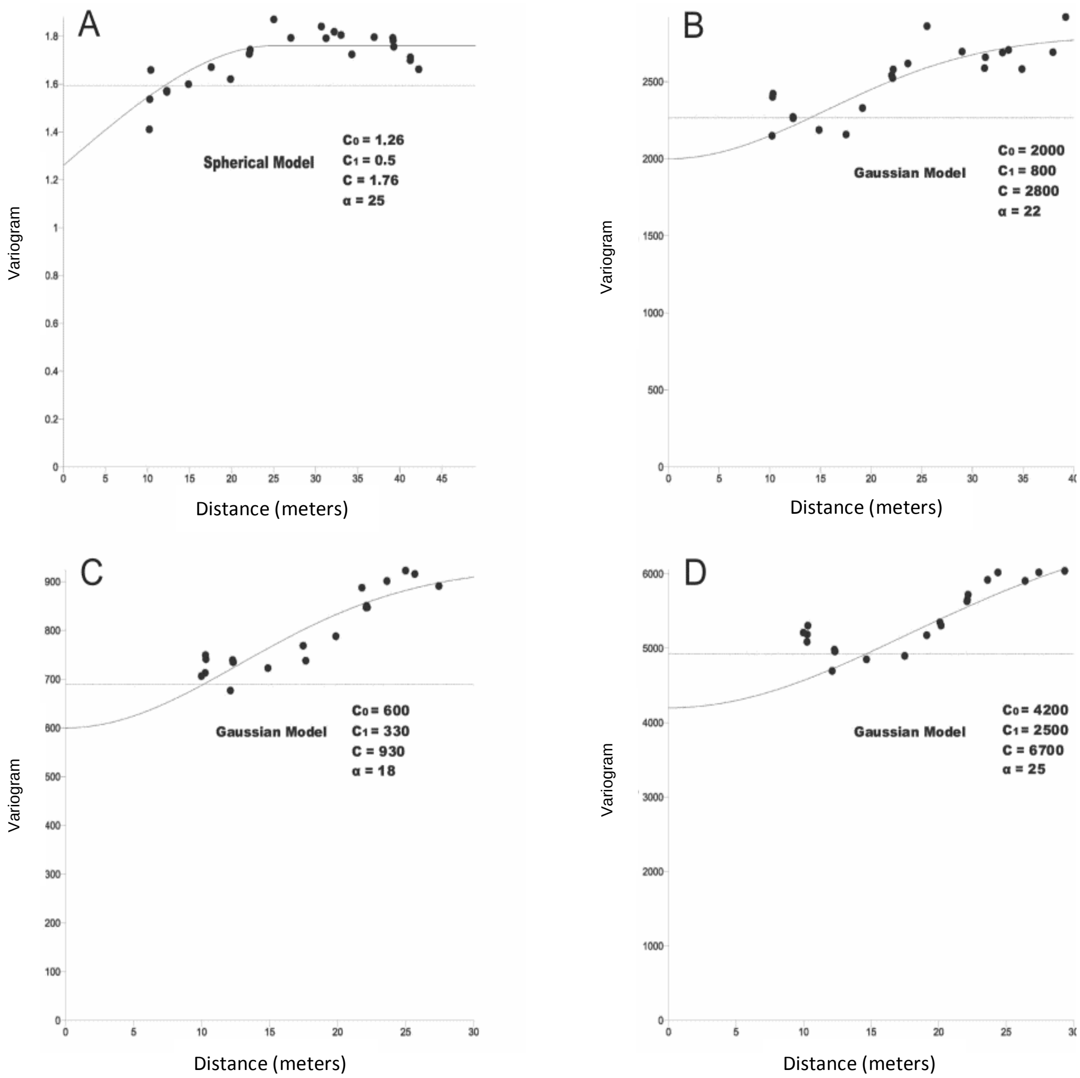

Figure 3. Semivariograms. (A) Number of parasitized larvae by *C. flavipes*. (B) Number of *C. flavipes* males that emerged in the second generation. (C) Number of *C. flavipes* females that emerged in the second generation. (D) Total number of *C. flavipes* adults that emerged in the first generation.

binomial distribution (Figure 2).

Fits of the negative binomial distribution to the data did not show a significant difference between the expected and observed frequencies, indicating that the spatial distribution of parasitism and number of insects emerged in the first generation was aggregated, independent of the sex of the parasitoids (Figure 2).

According to all the dispersion indices examined, the parasitism, number of males, number of females, and total number of emerged adults of *C. flavipes* indicated aggregated distribution, which was confirmed by adjustments to the frequency distribution data of the negative binomial distribution (Figure 2). Thus, the distribution of *C. flavipes* parasitism on *D. saccharalis* larvae was aggregated, indicating that the parasitoid did not disperse uniformly in the release area.

Geostatistical analyses

The semivariogram for *C. flavipes* parasitism showed a range of approximately 25 m. The range distance, defined as the distance between pairs, increased until a certain level (Figure 3A). The range indicates the flight distance of the parasitoid, or in other words, its dispersal potential. In this study, we found that *C. flavipes* parasitizes *D. saccharalis* larvae at distances up to 25 m from the release point (Figure 3A). This 25 m distance was the distance between the release point and the edge of the aggregation area.

Krigeage maps were generated using the data from the semivariograms with the objective of estimating the necessary interpolations to construct isolines and three-dimensional parasitism maps for *C. flavipes* (Figure 4A).

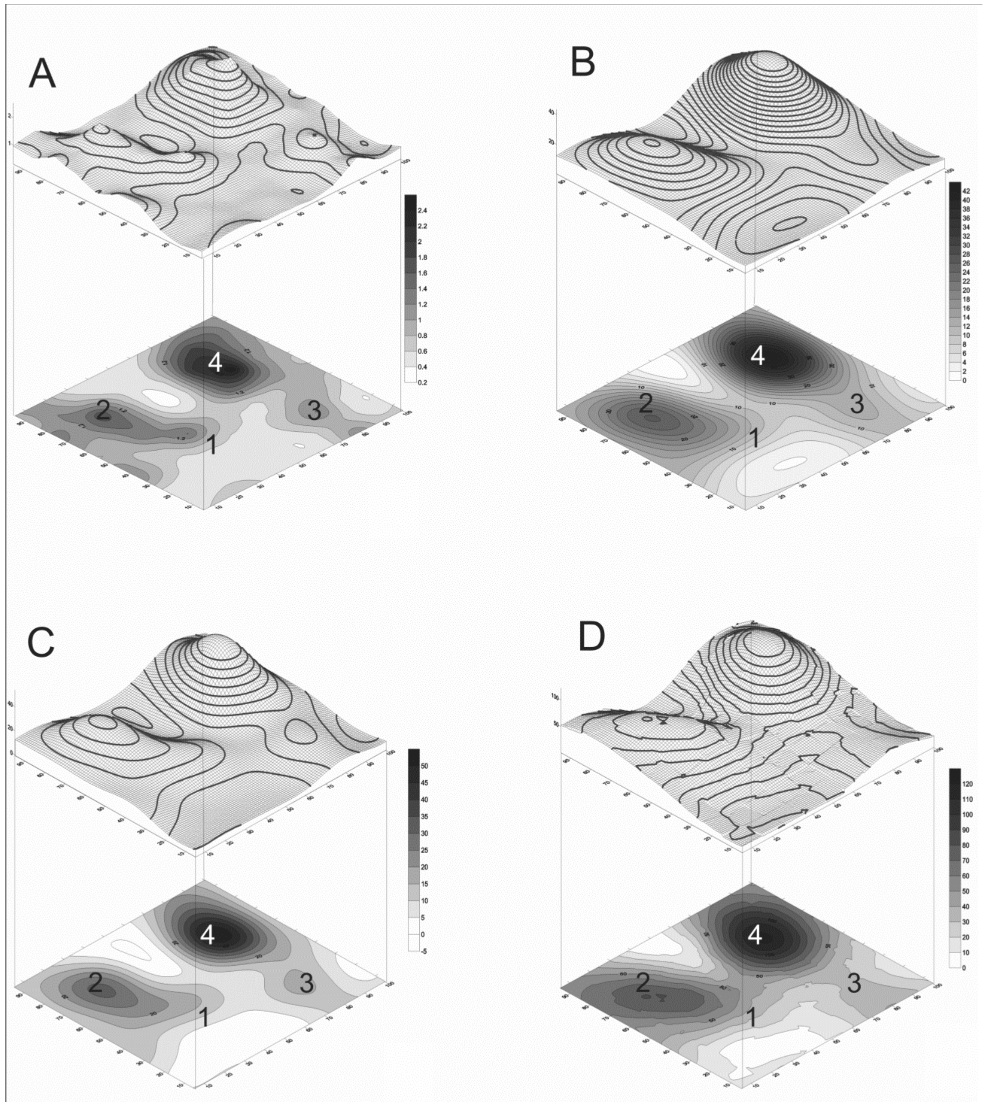

Figure 4. Krigeage maps. (A) Number of parasitized larvae by *C. flavipes*. (B) Number of *C. flavipes* males that emerged in the second generation. (C) Number of *C. flavipes* females that emerged in the second generation. (D) Total number of *C. flavipes* adults that emerged in the first generation.

From the map, we observed that each release point showed different parasitic behavior of *C. flavipes*. For better understanding, the release points are discussed separately. Release points one and three were less efficient than points two and four because they were located close to a dirty road used to transport agricultural machines. The low efficiency can be explained by the fact that the part of the site containing release points one and three was less protected by the crop. The release points closer to the road were thus less efficient than those that were protected by the crop. The dirty road cannot be avoided when releasing the parasitoids since it provides necessary access to the release area (Figure 4A).

The parasitoids released at point two showed higher parasitism aggregation than those released at the points located nearest to the road (release points one and three); the number of parasitized larvae ranged between 1.2 and 1.4 near the release point. However, this number decreased by 50% at a distance of 10 m from the release point and reached a value of 0.2 at a distance of 15 to 25 m from the release point (Figure 4A).

The parasitoids released at point 4 showed the highest parasitism ratio, ranging from 2.2 to 2.4 parasitized larvae near the release point. However, at 15 m from the release point, this value decreased by 50%, ranging from 1.0 to 1.2, and beyond this distance, the parasitism ratio was 0.8 - 0.4 parasitized larvae (Figure 4A).

Thus, despite the activity radius of *C. flavipes* (25 m) being comparable to the distance between the release points of the parasitoids (50 m), the number of parasitized larvae rapidly decreased, reducing to 50%, when the distance from the release point reached 10 m (Figures 3A and 4A).

A three-dimensional map for the number of males that emerged in the first generation was constructed on the basis of the corresponding semivariogram, and it showed that the emerged males had a range of 22 m (Figures 3B and 4B). From this map, we could also observe that there were differences among the number of male parasitoids that emerged at the four points. These differences can be explained in terms of the differences in the number of parasitized larvae at the same points. The points with a smaller number of parasitized larvae had fewer emerged males (Figure 4B). Since the number of parasitized larvae decreased with increasing distance from the release point, there was a corresponding decrease in the number of emerged male parasitoids. Near release point 4, we observed the emergence of 40 males; however, at a distance of more than 10 m from the release point, this number decreased by 50% (Figure 4B).

The semivariogram for the number of females that emerged in the first generation showed that the females had a range of 18 m (Figure 3C). However, as the parasitoids dispersed further from the release point, there was a corresponding decrease in the number of parasitized larvae and consequently in the number of emerged females, similar to the emerged males (Figure 4C).

Comparison of the krigeage maps for the male and female parasitoids showed that the number of emerged males and females was similar, with a sex ratio of approximately 1:1 (Figures 4B and 4C).

Considering the total number of *C. flavipes* in the first generation (Figures 3D and 4D), the semivariogram showed that the adults had a range of 22 m (Figure 3D) and the distribution and density of the adults (Figure 4D) followed the same behavior showed to males (Figure 4B) and females (Figure 4C), but were higher when compared to males and females, since this figures shows a sum of the emerged males and females. However, the distribution of this generation is an import tool to know the contribution of the emerged adults to control the next generation of *D. saccharalis* in the area.

DISCUSSION

Tests with traps - Experiments one to four

Attraction to color has been demonstrated in insects (Romoser, 1981). Yellow is more attracted than other colors to *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae) (Udayagiri et al., 1997) and in general, yellow color attracts parasitoids (Hoelmer et al., 1998).

In a previous study using traps to capture Braconidae insects, Noyes (1989) tested five methods of sampling Hymenoptera (sweep-netting, Malaise trapping, yellow pan trapping, flight intercept trapping and canopy fogging), demonstrating that for most groups of this order, sweeping was the most effective single method of sampling. Malaise traps were also very effective in most habitats. Flight intercept traps were found to be an ineffective means of sampling populations of Hymenoptera. However, there are no reports on the effectiveness of this method for the collection of *Cotesia* species.

We tested the influence of frass dissolved in the solution used in Moericke traps on the attraction of the parasitoids, but obtained negative results. During foraging, parasitoids utilize plant volatiles to locate their host habitat (Finidori-Logli et al., 1996). This technique was observed by Boethel and Eikenbary (1986). They commented that parasitoids are initially oriented in response to the stimuli provided by the plant and then in the second stage respond to the stimuli provided by the host. Similarly, the frass dissolved in the solution could serve as a stimulus for attracting the parasitoids to the traps, but the traps themselves were not effective in attracting these parasitoids.

In the experiment using sticky traps, it was observed that the insects were not attracted by the color of the traps. The observation that different numbers of insects were collected in the traps set at three different heights and that the traps placed at the ground level were more efficient evidently shows that *C. flavipes* individuals were collected by trapping and that they flew at a low height.

With the method using stems, with each stem containing a single larva, we observed 100% parasitism in “Experiment four” (laboratory test), regardless of the position of the stem in the cage or the addition of frass in the opening made for the insertion of the larva. Considering this result, we adopted the same method to test spatial distribution (Experiment five), using stems inserted upright in the ground, with the head of the larvae facing upward, and without the addition of frass in the opening made for the insertion of the larva.

The method of the use of larva with their head facing upward and no addition of frass was adopted to mimic the natural behavior of *D. saccharalis* larva and to prevent attraction by frass in the opening, the factor that can mask the results, interfering with the insect behavior.

Experiment 5

Aggregation indices and probabilistic models

Some parasitoids aggregate in patches with a high host density. Positive and negative relationships between parasitism ratios and host densities in different patches indicate direct and indirect relations of the patterns of parasitism with spatial density, respectively. When parasitoids and hosts are not spatially related, the parasitism pattern is independent of the spatial density

(Hassell, 2000). Parasitism pattern that is directly dependent on density is very important as a stabilizing factor for the host population, because it can reduce host population densities (Giles et al., 2000).

This differential spatial distribution of natural enemies and their hosts has been described by Kring and Gilstrap (1983). They demonstrated that the aphids *Schizaphis graminum* (Rondani) and *Rhopalosiphum padi* (Linnaeus) show an aggregated distribution in wheat crops in the United States, whereas their parasitoids *Lysiphlebus testaceipes* (Cresson), *Diaeretiella rapae* (M'Intosh), and *Aphelinus nigritus* (Howard) disperse randomly in the crops. However, in the aforementioned study, the parasitoids were not released but were natural populations with low population densities, which explain the random distribution pattern.

In the field, different natural hosts are present per sample point. The method of artificial infestation of the host, as adopted in this study, eliminates the variation in the host density per sample point because of the uniform distribution of the hosts.

Spatial distribution of *D. saccharalis* is aggregated and tended to be weaker (more random) at densities near the threshold spray in Florida (Hall, 1986) and ramdomly in sugarcane in Loissiania (Schexnayder Jr. et al., 2001). In the present study, the parasitism of *C. flavipes* was aggregated, but its host *D. saccharalis* was uniformly distributed in the field. This indicates that *C. flavipes* parasitism does not depend on host density.

Thus, the method using artificial introduction of *D. saccharalis* allows studies on the dispersion of the adult parasitoid only, without any interference of the host, thereby eliminating the interference of this variable.

Geostatistical analyses

Since determining the density of released parasitoids, number of release points and distribution of the releases is a very difficult problem (van Lenteren and Tommasini, 2003). There is no information about distribution of *C. flavipes* to determine number of releases. In the present study, a distance of 25 m from the original release point represented the limit of successful *C. flavipes* parasitism (Figure 4A). This distance is smaller than that reported by Botelho et al. (1980), who concluded that *C. flavipes* has the potential to disperse approximately 34 m. Further, it has been reported that this parasitoid can disperse to a distance of 64 m to find a host (Sallam et al., 2001).

In our study, release points two and four were more efficient because they were protected by the sugarcane crop in all directions, which provided shelter to the parasitoids. Further, these points were located far from the road, which could be a means by which the parasitoids could leave the sugarcane crop (Figure 4A).

Considering density of infestation of *D. saccharalis* around 3,000 larvae per hectare, generally found in sugarcane fields in the same area, there would be approximately one larva every two linear meters. Based in this study, releasing *C. flavipes* using the method with four points per hectare and 6,000 parasitoids, the parasitism performance will reach less than one parasitized larva (Figure 4A) and in this way, the control of *D. saccharalis* will not be satisfactory.

The fact that the *C. flavipes* strains used by Botelho et al. (1980) dispersed 34 m from the release point indicates that in current times, after 30 years, *C. flavipes* may have lost the genetic characteristics associated with aggressiveness and dispersal potential. This finding warrants additional dispersal tests to identify a release method using which the parasitoids could cover the entire area of 1 ha.

Assefa et al. (2008) identified genetic differences between *C. flavipes* strains, when comparing one from Ethiopia with other strains from different parts of the world, including a Brazilian strain from Piracicaba, Sao Paulo, Brazil. They identified nine different haplotypes and concluded that the African strains are similar to those from Piracicaba and Jamaica (haplotype I). The haplotypes could differ because of reproductive isolation over several generations and could differ with respect to the host-searching capability, parasitism, and flight range, which are important characteristics of biological control agents. Thus, we believe that the genetic differences between wild populations and the *C. flavipes* strains actually used, due to reproduction isolation and genetic degeneration can be linked to the aggressiveness of the *C. flavipes* strains.

Comparison of the krigeage maps for the emerged males and females showed that the number of emerged males and females was similar, with a sex ratio of approximately 1:1. Studies conducted by Suzuki and Iwasa (1980), Werren (1980) and Godfray (1994) show that sex ratio is a valuable index when investigating the hypothesis of local competition for copulation. Parasitoid females deposit lesser eggs in already parasitized larva and that the sex ratio of the offspring tends to become skewed toward a higher proportion of males. This suggests that when a female finds a host that is not adequate for the development of its offspring, it tends to produce male progeny. As adults, these males would copulate with many females (given that they are polygamous), increasing the female progeny responsible for superparasitism in the next generation.

This information and the finding that the observed proportions of the males and females in the present study were similar suggest the absence of superparasitism, as also indicated by the sex ratio close to 1:1. The lack of superparasitism indicates that the *C. flavipes* populations that occur naturally in the field (that is, insects that are not present because of releases) are either small or non-existent.

It appears that the results of the present study were not influenced by the presence of naturally occurring

C. flavipes in the area. This is because the presence of these parasitoids would tend to have produced a sex ratio skewing toward more males than females and also because the observed parasitism was aggregated. We believe that if there was a natural population of *C. flavipes* in the field, parasitism would have been observed outside the aggregation area. Interestingly, a mean of one to six *C. flavipes* releases are performed in the same area every year, implying that *C. flavipes* does not persist in the ecosystem after release.

C. flavipes parasitism on *D. saccharalis* larvae showed an aggregated distribution in the sugarcane plot used in this study. The aggregation radius of *C. flavipes* was 25 m. The method of using the natural host of *C. flavipes* in sugarcane stems proved to be efficient for assessing parasitoid dispersion. It is suggested that geostatistical analyses, which allow the evaluation and mapping of the number of parasitized larvae and the numbers of male and female parasitoids that emerged in the next generation can be useful for any study aimed at determining an efficient method for *C. flavipes* release.

The methods of the use of Moericke traps of different colors, addition of frass, and use of adhesive traps were not effective for capturing the parasitoids and therefore not appropriate for studies about cover area of *C. flavipes*.

The method using stems is appropriate to be adopted for sugarcane crops, allowing measuring the parasitism and studies on cover area of the parasitoid, its qualitative characteristics, number of release points per unit area, and other parameters. On the other hand, *C. flavipes* did not show any direct density-dependent relationship with *D. saccharalis* densities (Rossi and Fowler, 2003b). Sugarcane plants can support different cuts, or be destined for irrigation or seedling production. The harvest adopted extends over approximately 5 months per year. In this way, plants would not be of the same age across the smaller spatial scales during the growing period and all these factors may lead to different levels of *D. saccharalis* infestation, either spatially or temporally (Mailafiya et al., 2010; van Lenteren et al., 2003). In this way, studies to find a release method under different field conditions may contribute to manage this pest.

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Full Length Research Paper

Saved barley (*Hordeum vulgare*) seed quality in mid-altitudes and high-lands of Southern Ethiopia

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Samples of saved barley (*Hordeum vulgare*) seed were collected at sowing and threshing time from mid-altitudes and highlands of southern Ethiopia in 2009, and were examined in the laboratory and field condition to determine the status of seed quality. The seed samples included farmers' cultivar 'Horsiso' (two-rowed), 'Nuro' and 'Melo' (six-rowed). This study revealed that saved barley seeds were not vulnerable to insect pests. However, *Alternaria*, *Aspergillus*, *Cladosporium*. *Epicoccum*, *Fusarium*, *Helminthosporium*, *Penicillium*, *Trichoderma*, *Trichothecium* spp. and Ustilago hordei were detected. The seed samples collected at sowing showed lower standard germination (SG) and higher electrical conductivity (EC). All cultivars had SG above 80%, nevertheless field emergence index (FEI) showed 15.22 up to 37.35%, which is less than the ideal plant population. This indicates differences among cultivars in seed quality deterioration during storage. SG was positively correlated (r = 0.678) with field emergence (FE); EC showed a negative correlation with FE (r = -0.347) and SG (r = -0.233). In conclusion, farmers' saved seed was found to be low in quality. Therefore, farmers' seed management practices need to be improved to retain the seed quality and enhance productivity.

Key words: Field emergence index, fungi, germination, seed health.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important staple food crops in mid-altitudes and highlands of Ethiopia. Barley grain is used for the preparation of different foodstuffs in the country, such as malt products, *injera*, porridge, *qolo*; and local drinks, such as *tela*, *borde*, and beer. The straw is used as animal feed, especially during the dry season. It was cultivated on around one million hectares in the peasant private holdings in 2007/2008 in the main cropping season and the grain yield was 1376 kg per hectare (CSA, 2008). Barley is cropped twice a year: in the major season, which relies on June to September '*meher*' and March to May '*belg*' rainfall season. Diseases, insect pests, weed competition low-yielding varieties, and unimproved farming system are among the most important factors that reduce grain yield of barley in Ethiopia.

The extent of the formal seed system, particularly for barley is negligible in mid-altitudes and highlands of southern Ethiopia. The previous report indicated that informal seed system in Ethiopia was estimated at 80 to 90% (Bishaw et al., 2008). Moreover, commercial seed producers, as businessmen, they are mainly looking for profits so that they focus on a hybrid open-pollinated and other profit-generating crops only. Hence, they may not satisfy the demand for seed of unprofitable crops. Therefore, the informal seed system needs to be supported by better farming practices, especially for self-pollinated crops like barley. In addition, farmers use traditional way of farming practices. For instance, farmers do not keep barley seed separately, but simply draw the

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seed from the large quantity of grain stored for food and sow such a seed by broadcasting. Thus, the status of saved barley seed quality in mid altitudes and highlands of southern Ethiopia is not known. A seed lot possesses a high field planting value may lose slowly or rapidly its planting value during storage depending on storage conditions (Thomson, 1979).

In general, informal seed system and traditional way of farming system might be among most determinant factors for the barley grain yield reduction in the study area. Thus, to prioritize the determinant factors for the barley grain yield reduction and take appropriate measures, determination of saved barley seed quality status is among crucial actions. Therefore, the present study was conducted in Bore and Sora districts in southern Ethiopia in 2009 to assess the status of saved barley seed quality.

MATERIALS AND METHODS

Farmers' saved barley seed samples were collected from mid-altitudes and highlands of southern Ethiopia in 2009. The study was conducted in Bore district and Sora district. Formerly Bore district and Sora district were administered in the name Bore district. In this study Bore district refers both Bore and Sora districts. The altitude of the study area ranges from 1450 to 2900 m.a.s.l. The annual temperature ranges from 10.1 to 20°C. Barley (cultivar 'Nuro') is likely the dominantly grown crop in the district.

Seed sampling

Criteria-based purposive sampling was used to select the peasant associations (PAs). Eleven PAs each in the highland and in the intermediate categories were selected based on the accessibility and the extent of barley production. Four farmers from each 10 PAs and five farmers from one PA a total of 45 farmers were randomly selected in the highlands of the district. Similarly, 45 farmers were selected from the intermediate agro-ecology of the district, making ninety randomly selected farmers in the district as a whole.

All barley cultivars in the study area were distinctly named and grown for food. The six rowed barley, named '*Nuro*,' is the dominantly grown cultivar in the district. '*Horsiso*' (two-rowed, mostly used as roasted grain, locally called (*qolo)* and '*Melo*' (six-rowed) were the second predominantly grown cultivars in the highland and in the intermediate areas, respectively. Since *Nuro* was dominantly grown in both highland and intermediate agro-ecologies, it was selected for seed quality assessment along with *Horsiso* and *Melo*. But this cultivar was evaluated as *Nuro* (highland) and *Nuro* (intermediate) to differentiate the site of production. Thus, 23 seed samples of *Horsiso* and 22 seed samples of *Nuro* were collected from the 45 farmers selected in the highland area of the district. Likewise, 23 seed samples of *Melo* and 22 seed samples of *Nuro* were collected from 45 selected farmers in the intermediate area. *Nuro* cultivar seed samples collected from the highland and intermediate were separately bulked and analyzed. This was because the cultivar was grown at different agro-ecologies and also the seed management practices were expected to be different at different agro-ecologies. During seed sample collection, one kilogram of farmers' saved barley seed was taken from each selected PA and farmer. Seed sampling was made from different parts (store depths and points) of each seed container. A total of 90 kg seed samples (that is, 45 kg seed from each of highland and intermediate agro-ecologies) were collected at the time of sowing. Similarly, the same amount of seed sample was collected, at the time of threshing, from farmers addressed earlier in each agro-ecology. Thus, a total of 180 kg of seed samples was collected in the 2009 main cropping season. The same cultivars were bulked and eight composite samples were made from two cultivars each from highland and intermediate agro-ecologies at the time of sowing and at threshing. The seed samples of the highland and the intermediate cultivars separately collected from both agro-ecologies at the time of sowing and threshing were also separately examined and analyzed. Sub-sampling for obtaining working sample was made using rotary seed divider after mixing completely.

Insect pest assessment

Four replications of 100 seeds, each were taken at random as working samples and each was examined under the stereoscopic microscope for the presence of any insect pests. The injured seeds were counted and expressed in percentage.

Disease causing fungi examination

Seed-borne microorganisms were examined using the seed washing method (ISTA, 2008). The working seed samples were immersed in water containing Tween 20 as a wetting agent, and were shaken with a mechanical shaker to remove fungal spores and hyphae intermingled with or adhering to the seed surfaces. The supernatant was then separated by centrifugation (3000 revolution per minute for ten minutes) and removed by pouring off and the extracted material (residue) was examined under the compound microscope.

Seed-borne microorganisms were also examined using the agar plate method (ISTA, 2008). Seeds were surface-sterilized in 10% solution of commercial sodium hypochlorite (NaOCl), laundry bleach, for three minutes and rinsed several times with sterile distilled water. Then 15 seeds were evenly placed on potato dextrose agar (PDA) in nine cm diameter Petri-dish and incubated at 25°C with 12 h alternating cycle of light and darkness for seven consecutive days. Then, the associated microorganisms were observed under the compound microscope and identified.

Disease-causing bacteria examination

Twenty-five seeds were taken at random from each cultivar seed samples collected at sowing and threshing time. Seeds were surface-sterilized in 10% solution of commercial sodium hypochlorite (NaOCl) laundry bleach for five minutes, rinsed several times with sterile distilled water, placed on sterile paper towels to dry and incubated on PDA medium at 28°C for five days. Then the bacterial colonies were sub-cultured on standard media, namely nutrient glucose agar (NGA) and yeast extract–dextrose (YDC) containing $CaCO_3$. Colonies were directly visually examined for their color and also used for further tests. King's medium B agar (KB) and D-1 agar were used as selective media. Gram-staining and anaerobic growth tests (Hugh and Leifson, 1953) were also employed to identify seed-associated disease causing bacteria at the genus level.

Seed moisture content determination

Constant-temperature oven method was used to determine the moisture content (MC) of the seeds. All materials and procedures of the International Seed Testing Association (ISTA, 2008) including 0.2% replicates tolerance was applied. The MC as percentage by weight was calculated to one decimal place for each seed sample using the formula:

$$MC = \frac{M2 - M3}{M2 - M1} x100$$

M_1 is the weight in grams to three decimal places of the container and its cover; M_2 is the weight in grams to three decimal places of the container, its cover and seed sample before drying, and M_3 is the weight in grams of three decimal places of the container, its cover and seed sample after drying.

The standard germination

Farmers' saved barley seeds were tested to determine the maximum germination potential of farmers' saved barley cultivar seeds and to estimate the field planting value. Seed germination testing procedures and seedling evaluation were employed as specified by ISTA (2008). Four hundred seeds were randomly taken and seeded in four replicates (two paper towel rolls containing fifty seeds each making a replicate) with 100 seeds each. Seed samples collected at harvesting time were preheated at 30°C with free air circulation for seven days before placing under prescribed germination conditions to break seed dormancy. However, seed samples collected at sowing time were not preheated. The seeds were germinated between papers (BP) in towel rolls. The adjacent seeds were spaced sufficiently far apart. Towel rolls and the transparent plastic bags were loosely wrapped to allow sufficient air circulation around the seeds. The sown seeds were then incubated in germination cabinet at 20°C and 95% relative humidity for seven days. The plastic bags were illuminated from artificial light sources in the cabinet for eight hours per day.

Normal seedlings were daily removed starting from the fourth day. Badly decayed seedlings were removed but abnormal seedlings with other defects were allowed to remain on the substrate until the final count. Finally, seedlings were categorized into normal seedlings, abnormal seedlings, ungerminated seeds and dead seeds. The percentage of the number of normal seedlings, abnormal seedlings, ungerminated seeds and dead seeds were calculated to the nearest whole number. To check the reliability of a test result, the average percentage of the replicates were calculated and compared with tolerance ranges.

Electrical conductivity test

Four replicates of fifty seeds each were soaked into a 200 ml Erlenmeyer flask containing 75 ml of distilled and deionized water. The flasks were covered with paraffin to reduce evaporation and placed in an incubator at 25°C for 24 h. The distilled and deionized water was used as a control for the test. The electrical conductivity of the seed leachates was measured to determine seed vigor using electrical conductivity meter and expressed as $\mu S\ cm^{-1} g^{-1}$ according to seed vigor testing handbook (AOSA, 1983).

Field emergence index (FEI)

All barley seed cultivars collected at the time of sowing and threshing from both agro-ecologies were used for field emergence test. Seed samples collected at the time of threshing were stored for three months until seeds fully developed. Four replications of one hundred seeds each were sown in the Haramaya University research field. Emerged seedlings were daily counted until no more seedlings emerged. The field emergence index for each seed sample was calculated on the basis of the number of emerging seedlings following the procedure used by Egli and Tekrony (1995; 1996):

$$FEI = \frac{FE}{SG} x100$$

Where: FEI = Field emergence index; FE = mean seedling field emergence; SG = mean standard germination (SG).

The higher the value in FEI, the better the field conditions would be until FEI becomes equal to one, implying that it is ideal when FE is equal to SG.

Data analyses

Statistical analysis system (SAS version 9.0) software of the general linear model (GLM) procedure was applied to calculate seed quality data. The results of the statistical analyses were declared significant at P = 0.05. Mean separation was carried out using the least significant difference (LSD). Analyses of correlation coefficients were calculated using statistical package for social science (SPSS) software version 16.

RESULTS

The examination of seed for evidence of insect pests, pathogens and seed vigor elucidates the status of seed quality and performance of crop stand establishment in the field. Healthy seed with high physical purity, germination, and vigor implies the production of healthy seedlings, good crop stand establishment and thereby high yield. Prior seed health testing and other seed quality test results are indicators for the field establishment potential of the seed.

All examined saved barley cultivars seed samples revealed no evidence of insect pests infestation and seed damage. However, Fungi found the abundant microflora in tested barley seed samples. The fungi genera found at various levels of incidence in association with examined barley cultivars (Figure 1). The disease incidence difference could be due to differences in moisture content and seed management including differences in seed storage conditions.

Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium, Helminthosporium, Penicillium, Trichoderma, and Trichothecium spp. were seed microflora isolated from barley seeds (Figures 2 to 10). Plates of Microflora Isolated from Saved Barley Seed; plate size 6.25 by 6.25 centimeter.

In addition, washings of all seed samples taken at sowing and threshing time revealed infection by Ustilago hordei, which is the cause of covered smut of barley. Seed moisture content determination reveled that all seed samples collected at the time of sowing had a moisture content below 12.5%, cited which is the maximum level in the Ethiopian seed quality standard (certified seed D class), whereas samples collected at

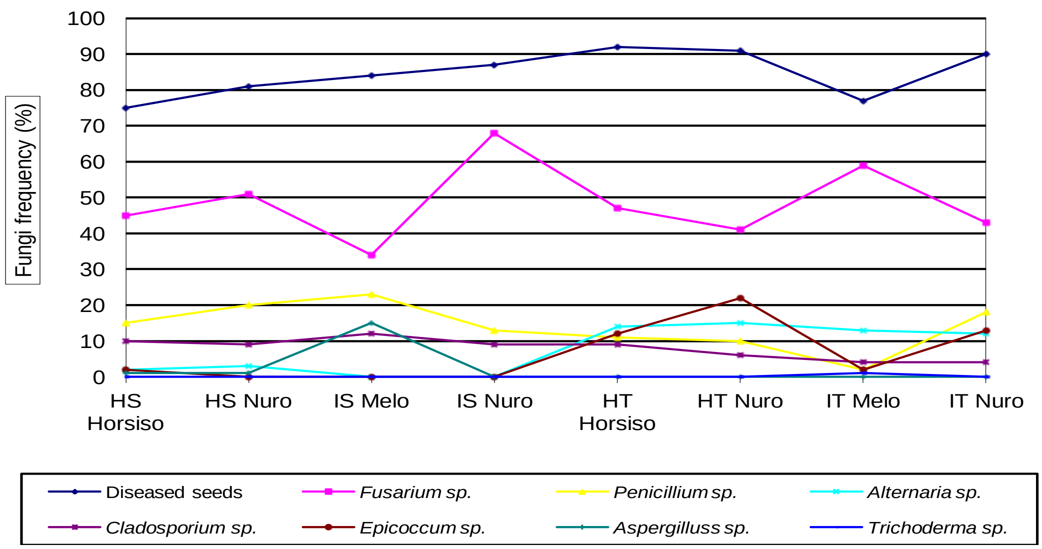

Figure 1. Incidence of fungi isolated from farmers' saved barley seed. HS and IS, samples collected at sowing time from highland and intermediate agro ecologies, respectively; HTand IT, samples collected at threshing time from highland and intermediate agro ecologies, respectively.

Figure 2. Microbes grown on agar plates.

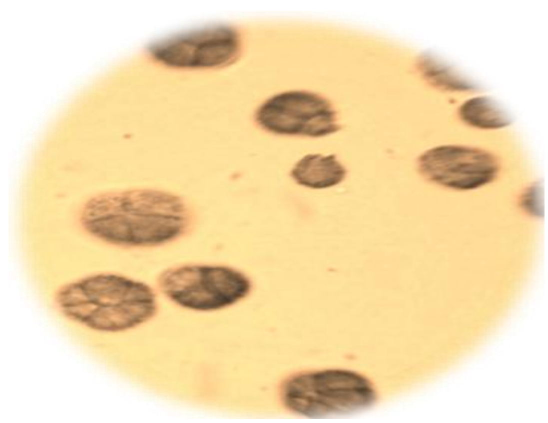

Figure 4. *Epicoccum* sp.

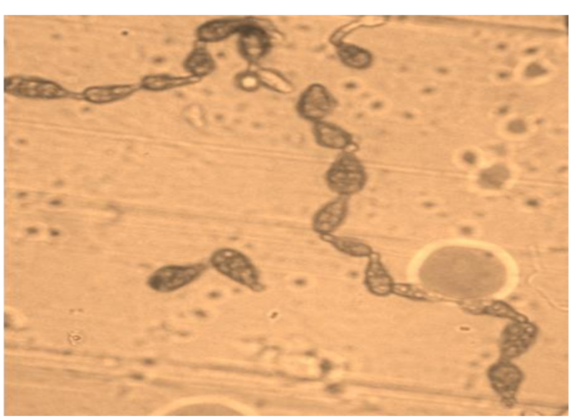

Figure 3. *Alternaria* sp.

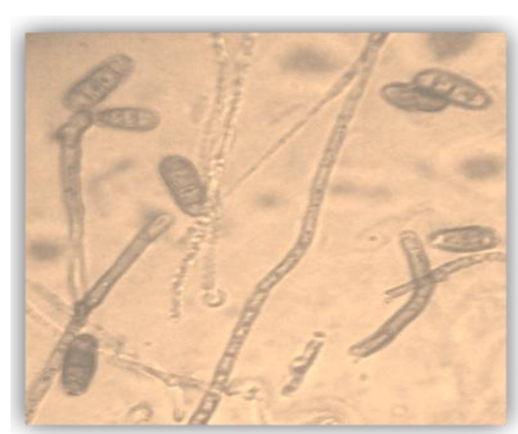

Figure 5. *Helminthosporium* sp.

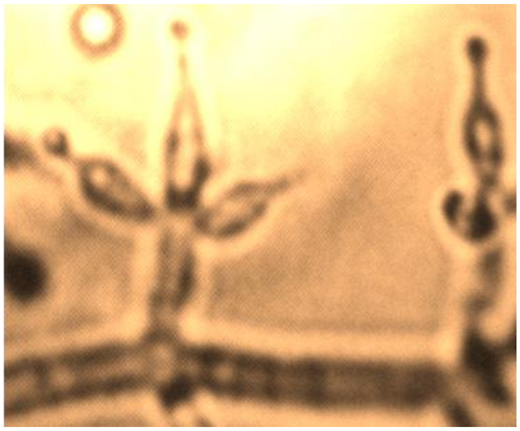

Figure 6. *Trichoderma* sp.

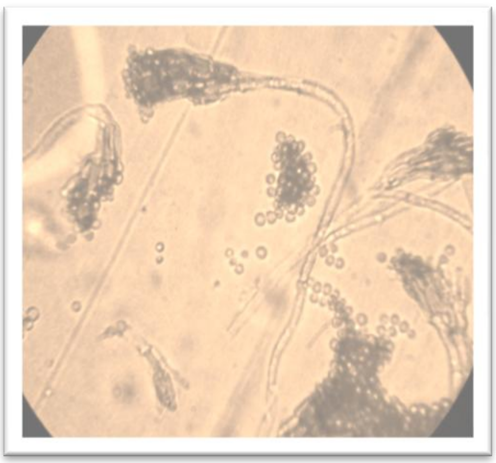

Figure 7. *Penicillium* sp.

Figure 8. *Trichothecium* sp.

Figure 9. *Aspergillus* sp.

Figure 10. *Fusarium* sp.

threshing time from both highland and intermediate agro-ecologies showed higher moisture content than the Ethiopian seed quality standard (Table 1). The more the seed moisture content, the more will be the seed quality deterioration.

DISCUSSION

Among various genera of fungi isolated from farmers' saved barley seed, *Fusarium* was the predominantly found disease-causing microorganism (Figure 1). The co-occurrence of seed microflora was observed in all seed samples except in *Horsiso* and *Melo* cultivar seed

Table 1. Mean moisture content, germination and field emergence of farmers' saved barley seed.

Sample †	MC (%)	SG (%)	EC (μ cm^{-1} g^{-1})	FE (%)	FEI (%)
HS *Horsiso*	11.97^{d}	89^{a}	52.72^{a}	59.0^{dc}	66.29
IS *Melo*	11.31^{f}	83^{b}	30.90^{cd}	52.0^{d}	62.65
IS *Nuro*	11.61^{ef}	90^{a}	33.31^{bc}	76.0^{ab}	84.44
HT *Horsiso*	13.64^{a}	92^{a}	30.40^{cd}	65.0^{c}	70.65
HT *Nuro*	13.53^{a}	91^{a}	19.09^{f}	67.0^{bc}	73.63
IT *Melo*	12.61^{c}	90^{a}	27.12^{de}	58.0^{dc}	64.44
IT *Nuro*	13.02^{b}	92^{a}	23.39^{ef}	78.0^{a}	84.78
Mean	12.42	89.63	31.63	64.38	
CV (%)	1.09	3.48	10.13	9.94	
SE±	0.22	0.69	1.77	1.81	

Figures followed by the same letter in the column are not statistically different from each other at 0.05 probability level. † HS and IS, samples collected at sowing time from highland and intermediate agro ecologies, respectively; HT and IT, samples collected at threshing time from highland and intermediate agro ecologies, respectively; mean MC, moisture content; mean SG, Standard germination; mean EC, electrical conductivity; mean FE, field emergence; FEI, field emergence index; CV, Coefficient of variation; .SE, Standard error of means.

samples collected at the time of sowing, and *Nuro* (intermediate) collected at threshing time. Profuse development of fungi could be an indication that the seed is not of good quality (ISTA, 2008).

Nuro cultivar collected from intermediate agro-ecology at sowing and threshing time was positive for *Xanthomonas* and *Pseudomonas* spp. Similarly, *Melo* seed taken at threshing was found to be positive for same bacteria. This indicated that bacterial infection was not common in all barley seed samples as compared to fungal infection. This observation is consistent with the previous finding (Mundt and Hikle, 1976).

The seed moisture content above the standard could contribute to seed quality deterioration during storage time. This study showed that the higher the moisture content, the lower was the field emergence. This is due to increase in fungal growth under the moist condition (Harpper and Lynch, 1981).

The seed samples collected at sowing showed a lower SG and higher EC than samples collected at the threshing time when the same cultivar was considered. However, all seed samples had germination above 80%, which is the minimum seed quality standard of Ethiopia (Table 1). Nevertheless, SG does not always indicate the potential of seed lot performance in less optimal field conditions (Hampton and Tekrony, 1995). The higher EC value implies reduction in seed vigor due to an increment of membrane permeability (Vieira and Krzyzanowski, 1999). Samples of a cultivar collected at threshing time revealed better field emergence than samples taken at the time of sowing. Moreover, FEI of the cultivars showed 15.22 up to 37.35% less than the ideal plant population in the field (Table 1). This fact could be explained by the differences among cultivars in seed quality deterioration during storage.

In this study, it was found that SG was significantly and positively (r = 0.678) correlated with FE, whereas EC showed a non-significant negative (r = -0.347) correlation with FE and SG (r = -0.233). This result is consistent with previous reports on barley and other crops (Kim et al., 1989; Krzyzanowski, 1999).

In general, farmers' saved seed was low in quality because of high seed moisture content at the time of threshing and traditional seed management practices, leading to profuse growth of disease-causing fungi and bacteria. As a result, farmers' saved seed loses their germination and field emergence. This observation is consistent with seed germination reduction due to competition between micro-organisms and growing seed for oxygen (Mathews and Collins, 1975).

In conclusion, farmers' seed management practices required to be improved to reduce incidence of disease causing micro-organisms and seed infection, and thereby to enhance seed planting value and productivity. Further research required on seed storage methods and their contribution for the barley seed quality reduction.

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Yield, nutrient uptake and potassium use efficiency in rice fertilized with crushed rocks

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The increasing world population has led into big food and raw material demand, with high pressure on agriculture. In Brazil, potassium fertilizers are mostly imported rising interest on rocks and mining rejects as alternative sources. The objective of this work was to evaluate the effect of crushed rocks over mineral nutrition, yield and efficiency of potash fertilization in rice. The experiment was carried out under greenhouse conditions in pots with 3.7 kg of Oxisol soil samples. The experimental design was completely randomized, in a factorial layout 4 × 6, comprising six crushed rocks used as alternative multinutrient fertilizer (breccia, biotite schist, ultramafic, Chapada byproduct, mining byproduct and phlogopite), and four doses based on potassium (0, 200, 400, 600 kg K_2O ha^{-1}), with four replications. The concentration and accumulation of potassium, copper, zinc and nickel in rice shoot dry mass were determined. These data were used to evaluate the efficiency index of K-source rocks on fertilization. It was shown that crushed rocks promote alterations in mineral nutrition, grain yield and potassium use efficiency, remarking their potential to be used as alternative fertilizer in rice cropping systems.

Key words: Alternative nutrient source, micronutrient, fertilization efficiency, plant nutrition, soil conditioner.

INTRODUCTION

In Brazil, the first studies on materials with low nutrients dissolution kinetics were carried out in the 1970's. Since then, new technological methods have been tested in order to increase nutrient solubility from the variable content in rocks. Among these methods, crushing, thermal processes of melting, acidification and microorganisms inoculation were tested. In addition, high energy demand in processing the materials and the competition with potassium chloride. In recent times, the these rocks have been evaluated for direct use in agriculture. The methods to turn these rocks soluble have shown unfeasible due to their low nutrients release and Brazilian agricultural context has changed, as well as the consumption rates of potassium chloride (Lopes, 2005). The increase in potassium consumption by agriculture, leading to high importing dependence of K fertilizers and

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Table 1. Total content of K_2O, Na_2O, P_2O_5, CaO, MgO, Cu, Zn and Ni in crushed rocks[1].

Rock	K_2O[(2)]	Na_2O[(2)]	P_2O_5[(2)]	CaO[(2)]	MgO[(2)]	Cu[(3)]	Zn[(3)]	Ni[(3)]
	Percent (%)					Milligram per kilogram (mg kg^{-1})		
Breccia	2.18	0.31	0.94	9.03	7.09	59.9	128.7	73.9
Ultramafic	3.10	1.71	1.22	13	18.50	87.4	113.1	651.9
CBP[(4)]	3.39	1.62	0.19	3.19	3.88	437.5	123.0	2.8
MBP[(5)]	11.80	0.72	0.42	3.58	0.70	816.8	28184	380.3
Biotite Schist	2.07	0.86	0.06	5.27	13.8	9.9	290.5	146.4
Phlogopite	7.71	0.16	0.2	0.98	22.89	9.1	902.7	1425.2

[(1)] Rocks crushed at 0.3 mm in this analysis; [(2)] method 4A and 4B of Acmelab (Canada) Laboratory which has as principle the sample fusion in lithium metaborate/tetraborate; [(3)] Method 3052 USEPA (1996); [(4)] Chapada byproduct; [(5)] mining byproduct.

the lack of sources to organic agriculture have risen interest on the use of crushed rocks (Bakken, 2000; Van Straaten, 2006).

Crushed rocks can be considered multinutrient fertilizers carrying silicate minerals containing other macro and micronutrients in variable concentrations. Among these, the main are phosphorus, calcium and magnesium (Wilpert and Lukes, 2003; Ribeiro et al., 2010). In addition to the supply of macronutrients, some crushed rocks promote changes in soil characteristics such as acidity due to variable Relative Neutralization Value (RNV). Crushed rocks must be applied in high amounts to supply macronutrients. Because of their variable composition, they also end up taking micronutrients to soil, which become available along crop seasons (Amaral-Sobrinho, 1992). Consequently, they may be found on parts of cultivated plants in areas where they were applied as alternative fertilizers.

Thus, it is necessary to know the potential of nutrient release, uptake and accumulation in plants. This study was carried out aiming to evaluate the effect of crushed rocks utilized as multinutrient fertilizer in terms of potassium supply and efficiency to rice crop nutrition.

MATERIALS AND METHODS

A greenhouse experiment was carried out at the Soil Science Department of "Universidade Federal de Lavras", Lavras, MG, Brazil, from November 2010 to April 2011. Samples of a sandy clay loam Oxisoil were collected in Itutinga, MG, under natural savannah vegetation, 0 to 20 cm from the surface. Soil was air dried, sieved with a 4-mm-mesh, homogenized and placed in 3.7 kg cultivation pots. Concomitantly, soil samples were collected for chemical and physical characterization, as follows: K = 22 mg dm^{-3}, S = 5.4 mg dm^{-3}, $P_{(Mehlich1)}$ = 0.9 mg dm^{-3}, Ca = 0.1 $cmol_c$ dm^{-3}, Mg = 0.1 $cmol_c$ dm^{-3}, Al = 0.1 $cmol_c$ dm^{-3}, H + Al = 1.7 $cmol_c$ dm^{-3}, SB = 0.3 $cmol_c$ dm^{-3}, t = 0.4 $cmol_c$ dm^{-3}, T = 2.0 $cmol_c$ dm^{-3}, Fe = 27.4 mg dm^{-3}, Zn = 0.5 mg dm^{-3}, Cu = 0.7 mg dm^{-3}, B = 0.0 mg dm^{-3}, Mn = 0.4 mg dm^{-3}, sand = 600 g kg^{-1}, silt = 170 g kg^{-1} and clay = 230 g kg^{-1}. The experimental design was a completely randomized in 4 × 6 factorial scheme with six crushed rocks utilized as alternative multinutrient fertilizer (breccia, biotite schist, ultramafic, mining byproducts, Chapada byproduct and phlogopite), and four doses based on potassium supply (0, 200, 400 and 600 kg K_2O ha^{-1}), with four replications (Table 1).

Most rocks were sampled in various mining sites, where they are usually discarded. Understanding their potential for agricultural use is advantageous, decreasing the environmental impact of mining waste to turn them into farming input. A brief description of each rock and respective mining site is presented as follows: Volcanic alkaline breccia, found in Santo Antônio da Barra, Goiás, Brazil was formed in volcanic conduits, being composed by feldspathoids, zeolites and volcanic glass. Alkaline-ultramafic is a mining byproduct from Lages, Santa Catarina, Brazil. The original rock is formed by igneous intrusion, composed by ferromagnesian minerals (olivine, pyroxene and phlogopite), plagioclase and carbonates. It occurs in an old mining place to produce building materials. Biotite Schist, named here as Chapada byproduct from Chapada, Novo Horizonte, Goiás, Brazil was formed by hydrothermal process, altering granitic rocks and generating copper and gold ore. It is composed by biotite, muscovite, having as accessories quartz and carbonates. This material was obtained from flotation and crushing process, not submitted to chemical transformation. Biotite schist, a byproduct of emerald ore from Itabira, Minas Gerais, Brazil, is formed by hydrothermal processes of granite fluid passage over ultramafic rocks that formed emerald. Composed by biotite and quartz, this material is accumulates as waste in mining sites.

Phlogopite schist, called phlogopite (phlogopitite, emerald mining by product from Campo Formoso, Bahia, Brazil). Rock formed from hydrothermal processes of fluids passage of granitic composition over ultramafic rocks that formed emerald; composed by phlogopite and serpentine. This material is an accumulated waste in emerald mining sites. Mining byproduct, manganese ore waste found in Belo Horizonte, MG, Brazil, originated from metallurgical process of manganese extraction. In processing, the potassium is separated from ore and concentrated on wastes. A more detailed characterization of these materials, including X-ray diffractometry, photomicrography, and effects over soil chemical attributes are available at Silva et al. (2012). The total amount of rocks, in t ha^{-1}, corresponding to doses of 200, 400 and 600 kg ha^{-1} K_2O equivalent, were respectively: Breccia 13.64, 27.28 and 40.92; ultramafic 9.59, 19.18 and 28.77; Chapada byproduct 8.77, 17.54 and 26.31; mining byproduct 2.52, 5.04 and 7.56; biotite schist 14.36, 28.72 and 43.08; and phlogopite 3.86, 7.72 and 11.58. The rocks were used in their original granulometry (Table 2), as they are likely to be applied in soils, without additional energetic costs on crushing. In addition to potassium, the sources utilized in this study are also micronutrient suppliers such as nickel, copper and zinc which were added to soil in variable amounts, according to treatments of K_2O doses (Table 3).

The value of water pH and relative neutralization value (RNV) shows that these products provide plants with K and micronutrients,

Table 2. Proportion of granulometric fractions of crushed rocks.

Rock	Proportion of rock particles by size				
	1 mm	0.42 mm	0.250 mm	0.125 mm	< 0.125 mm
	Percent (%)				
Biotite Schist	9.88	30.14	25.02	23.36	11.60
Phlogopite	11.55	27.43	35.76	13.62	11.64
CBP (1)	21.13	23.75	13.85	22.64	18.63
Breccia	32.85	31.69	9.28	22.01	4.17
Ultramafic	22.14	20.84	14.29	22.29	20.44
MBP (2)	26.58	28.34	8.60	32.42	4.06

(1) Chapada byproduct; (2) mining byproduct.

Table 3. Total amount of rocks and micronutrients (mg pot^{-1}) added by treatments.

Rock	200 kg de K_2O ha^{-1}				400 kg de K_2O ha^{-1}				600 kg de K_2O ha^{-1}			
	Dose	Ni	Cu	Zn	Dose	Ni	Cu	Zn	Dose	Ni	Cu	Zn
	g pot^{-1}	mg pot^{-1}			g pot^{-1}	mg pot^{-1}			g pot^{-1}	mg pot^{-1}		
Breccia	20.5	1.51	1.22	2.6	40.9	3.02	2.44	5.3	61.4	4.53	3.66	7.9
Ultramafic	14.4	9.37	1.26	1.6	28.8	18.7	2.52	3.3	43.2	28.11	3.78	4.9
CBP(1)	13.2	0.04	5.75	1.6	26.3	0.08	11.5	3.2	39.5	0.12	17.25	4.8
MBP(2)	3.8	1.44	3.09	107	7.6	2.88	6.18	213	11.3	4.32	9.27	320
Biotite Schist	24.5	3.59	0.24	7.1	43.1	7.18	0.48	14.3	64.6	10.77	0.72	21.4
Phlogopite	5.8	8.24	0.005	5.2	11.6	16.48	0.01	10.4	17.3	24.72	0.015	15.7

(1) Chapada byproduct; (2) mining byproduct.

Table 4. Rock pH[1], soluble CaO and MgO, calcium carbonate equivalent (CCE), fineness (F), calcium, magnesium and relative neutralization value (RNV)[2].

Rock	pH (H_2O)	CaO	MgO	CCE	F	RNV	Ca	Mg
		Percent (%)						
Biotite Schist	8.2	0.07	1.45	1.65	68.40	1.13	0.05	0.84
Phlogopite	8.5	0.13	0.57	0.45	82.32	0.37	0.09	0.34
MBP(3)	10.8	2.73	0.55	11.29	87.92	9.92	1.95	0.33
Breccia	8.3	2.23	1.34	4.78	71.20	3.41	1.59	0.80
Ultramafic	9.4	10.84	8.95	39.47	71.24	28.12	7.74	5.39
CBP(4)	7.6	2.07	1.62	5.99	98.32	5.89	1.48	0.97

(1)Moreira et al. (2006); 2Brasil (2007); (3) mining byproduct; (4) Chapada byproduct.

with potential to change soil acidity (Table 4). The pots containing treatments were sown with 10 rice seeds cv. Curinga on 11/19/2010. After emergence, exceeding seedlings were eliminated leaving two plants. The soil moisture was kept constant at filed capacity. Maintenance fertilization was done with 450 mg kg^{-1} P and 300 mg kg^{-1} N, split in three topdressings, and 50 mg kg^{-1} S, without micronutrients addition. The experiment was harvested 120 days after emergence, when plants were cut near to soil surface. From the harvested material, shoot and roots were collected and washed. All materials were placed in paper bags and dried at 75°C in forced ventilation oven until constant weight for dry matter and yield evaluations. Shoot and grain samples were milled separately and 2-g-samples were collected, and digested with nitric-perchloric acid to determine K, Ni, Cu and Zn content according to the method described by Tedesco et al. (1995). Nickel, Cu and Zn were determined by atomic absorption spectroscopy and K by flame spectrophotometry. The analysis of quality were based on National Institute of Standards and Technology (NIST) reference BCR® 414 – Plankton which defines the content limits for Ni (18.8), Cu(29.5) and Zn (111.6) in mg kg^{-1}.

The accumulation of K, Ni, Cu and Zn was determined on shoot dry matter and grains. Indexes of potassium fertilization efficiency were calculated for grain and shoot yield: i) agronomic potassium use efficiency (KUE) = (yield with K – yield without K): dose of K_2O;

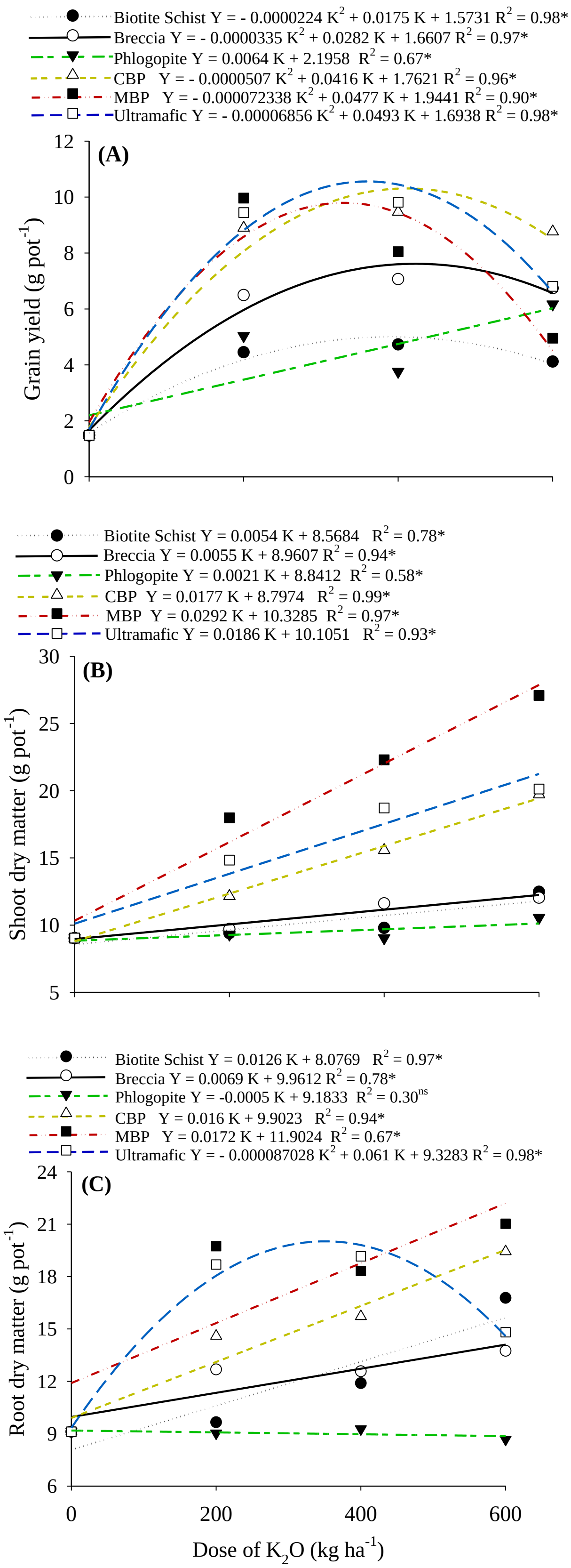

Figure 1. Effect of crushed rocks applied as equivalent K_2O dose on rice grain yield (A), shoot (B) and root dry matter (C).

and potassium recovery (KR) = K in shoot with K - K in shoot without K; dose of K_2O (Fageria et al., 2010). Analysis of variance utilized software SISVAR (Ferreira, 2011). For comparison between the effect of doses, rocks and their interaction, regression analyses were performed according to the best fit equations.

RESULTS AND DISCUSSION

There were significant differences ($p<0.05$) for rock dose, source and interaction dose × source for grain yield, shoot and root dry matter, K, Ni and Zn content and accumulation in rice shoot grains. Crushed rocks promoted increases on rice shoot and root dry matter as potassium doses increased, although the effect over grain yield was variable (Figure 1A, B and C). The highest rice grain yield was attained with ultramafic in the dose of 360 kg of K_2O ha^{-1} (10.56 g pot^{-1}), while the lowest occurred without crushed rocks application. Regardless of K_2O dose, biotite schist and phlogopite presented the lowest rice grain yield. Seed yield, regardless K_2O doses, reached maximum value with Chapada byproduct and ultramafic. The effect of potassium on yield of several rice genotypes indicated values between 10.07 and 16.37 g $plant^{-1}$, with genotypes average 13.62 g $plant^{-1}$ (Fageria et al., 2010). With this reference, on silicate rocks experiment, the ultramafic was superior to phlogopite and alkaline breccia and did not differ from potassium chloride (Barbosa et al., 2006). Rice shoot and root dry matter had a linear response to K_2O supplied by rocks. The exception was for rice root dry matter with ultramafic, where quadratic is attributed to yield decrease at the highest dose. This may be related to sodium excess present in the composition of ultramafic (Table 1). Once Na was released to soil, it may have caused cation unbalance and raised the salinity of solution, reducing root growth with negative effect on grain yield at 600 kg of K_2O ha^{-1} (Table 3).

Adverse effects of salinity and sodium saturation on rice root growth under different management of potassium fertilization was previously described (Carmona et al., 2009). The harmful effect of salinity on nutrient uptake was related to a chemical competition of Ca, Mg and K with Na. The increase in exchangeable sodium associated with ultramafic rock has been related to increasing proportion of Na in CEC, reaching up to 16% at 300 kg K_2O ha^{-1} rock equivalent (Resende et al., 2006; Ribeiro et al., 2010). The decreasing shoot and root dry mass production in the rock equivalent K_2O doses in this experiment were as follows: mining byproduct > ultramafic > Chapada byproduct > biotite schist = breccia = phlogopite. For every 100 kg of K_2O ha^{-1}, there was a response of 2.92 g pot^{-1}, on shoot dry mass production; while for phlogopite, this value was 0.21 g pot^{-1}, highlighting the difference among these alternative fertilizers to release nutrients and improve rice nutrition with positive effect over biomass production. The K content in rice shoot varied between 5.09 to 12.21 g kg^{-1}

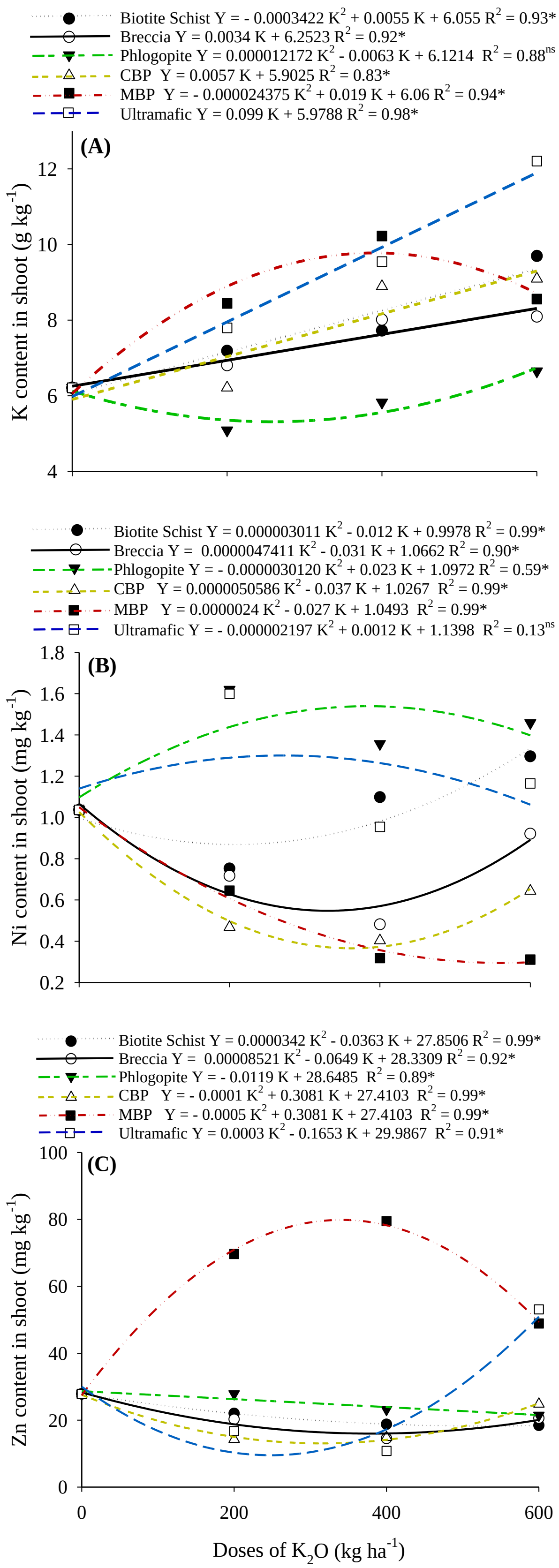

Figure 2. Effect of crushed rocks applied as equivalent K_2O dose on potassium (A), nickel (B) and zinc (C) content in rice shoot.

(Figure 2A), indicating a cycling effect when crop residues are returned to soil after harvest. It may be utilized by succeeding crops, on conventional and organic system for which available sources of potassium are scarce.

In general, K content in shoot increased proportionally to crushed rock dose. The highest grain yield, shoot and root dry matter was associated to increasing K from mining byproduct and ultramafic. The lowest content occurred when rice was fertilized with phlogopite. The nickel content in plant parts had differentiated effect with crushed rocks application (Figure 2B). In shoot it was high (Table 3), when ultramafic, phlogopite and biotite schist were used as K source. The ultramafic rocks present high total contents of Ni in its composition (Brady and Weil, 2002). The mineral dissolution however is variable and can influence Ni availability and all other nutrients present in the rock. Thus, it is relevant to study extractors that allow quantify available Ni, since the total content may not be released during the crop cycle. In mature leaf tissues of various species, Ni sufficiency ranges from 0.1 to 5 mg kg^{-1}, with toxicity varying between 10 to 100 mg kg^{-1} (Kabata-Pendias and Pendias, 2001). Therefore, in this study, the Ni in rice shoot was sufficient for plant nutrition. Another micronutrient released by these alternative fertilizers is zinc, which contents in rice shoots were in a range from 84.33 to 7.29 mg kg^{-1} (Figure 2C), in the dose of 275 kg K_2O ha^{-1} from mining byproduct and 308 kg K_2O ha^{-1} applied by ultramafic, respectively. Zn content in rice dry matter has been defined as adequate when reaches 67 mg kg^{-1}, while at 673 mg kg^{-1} it becomes toxic. Thus, among the selected materials, mining byproduct emerges as an alternative source of this micronutrient for rice cropping systems, besides the supply of K, Ni and Cu. Moreover, it must be pointed out that the need of Zn application in most Brazilian soils, due to low availability of this nutrient to crops (Oliveira et al., 2005). This finding strengthens the possibility of utilizing alternative sources that take also micronutrients, as Zn and in smaller amounts of Cu and Ni.

Nutrient content in shoot is a reference to assess plant nutrition although it may be inversely related to yield due to the nutrient dilution effect in plant. Two plants with the same nutrient concentration in shoot may have different yield, related to nutrient absorption and accumulation in diverse parts of the plant. To understand these effects, nutrient accumulation must be calculated to associate it to dry mass production and grain yield. In this work, K accumulation was higher when rice was fertilized with mining byproduct and ultramafic. The K accumulation increased with increasing doses of this macronutrient applied as crushed rocks (Figure 3A). Every 100 kg K_2O ha^{-1} of mining byproduct and ultramafic contributed to K accumulation in shoot of 35.45 and 38.01 mg pot^{-1}, respectively. The rocks promoting high K accumulation in shoot in decreasing order were: mining byproduct = ultramafic > Chapada byproduct > biotite schist = breccia

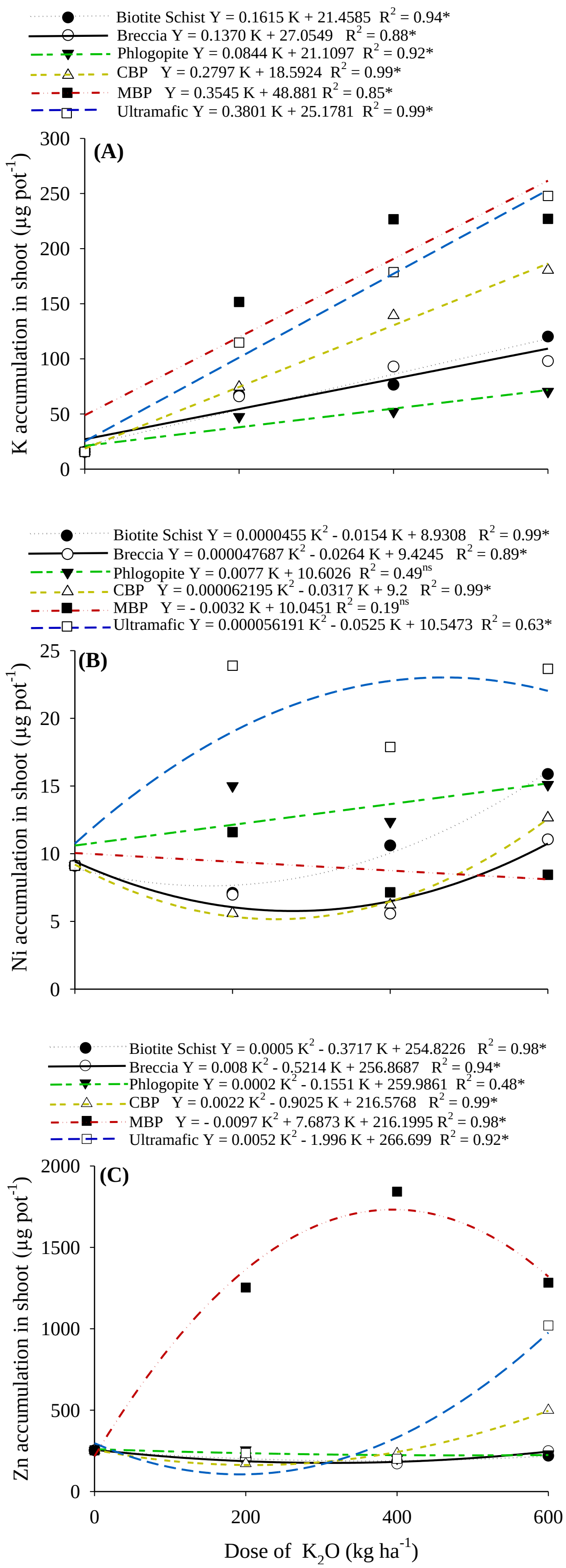

Figure 3. Effect of crushed rocks applied as equivalent K_2O dose on potassium (A), nickel (B), cooper (C) and zinc (D) accumulation in shoot.

> phlogopite. These differences in K content and accumulation in shoot shows the potential of K release of alternative fertilizers. Ultramafic, phlogopite and biotite schist differed from one another in Ni accumulation in rice shoot (Figure 3B). In mining byproduct, the element that accumulated most in shoots was Zn, differing from other rocks (Figure 3C). These differences among crushed rocks can be explained by the high total content of Zn in mining byproduct (Table 3), which is more available than the other rocks. Its noteworthy mentioning that crushed rocks were applied as potassium source, in large amounts, taking along variable levels of micronutrients, reflecting in the present results.

Crushed rocks affected significantly ($p<0.05$) Cu content and accumulation in rice shoot proportionally to the doses (Figure 4A and B). Moreover, the types of crushed rocks affected significantly only Cu accumulation in shoot (Figure 4C). Cooper content in rice shoot was higher in absence of crushed rocks. This could be ascribed to nutrient dilution, or high concentration in shoot of control (no crushed rock application) due to reduced biomass production. It can be seen that Cu accumulation, as related to dry mass production, increased with K_2O crushed rock equivalent (Figure 4B). For every 100 kg of K_2O ha^{-1} applied there was response in Cu accumulation in shoot of 3.66 mg pot^{-1}. Regardless of K_2O dose, for Cu accumulation, the rocks were sorted into two groups (Figure 4C). In the first group, with higher level in shoot were: Chapada byproduct, ultramafic and mining byproduct and, while in the second with lower Cu, accumulation was: biotite schist, phlogopite and breccia. Plant response was a function of rock quantity based upon K_2O content (Table 3). Nutrient accumulation in grains depends directly on its translocation in plant. Translocation can be defined as the ionic movement from the absorption site in root and transfer to other parts (Malavolta et al., 1997). In this sense, some nutrients may be translocated to edible parts, such as grains that if in excess, causes risk to human health (Martins et al., 2003; Rangel et al., 2006). Ni, Cu and Zn are in the group of elements that move freely in plant and enter the food chain.

The content and accumulation of K, Ni, Cu and Zn in rice grains increased with increasing K_2O doses applied as crushed rocks (Figures 5 and 6). In most cases, the content and accumulation of a chemical element in different plant parts is function of its availability and the content in plants increases with its concentration in soil (Gussarsson et al., 1995). The rocks which promoted highest K content and accumulation in grains were mining byproduct, ultramafic and Chapada byproduct (Figures 4A and 5A). It has been reported that most of absorbed K accumulates in shoot and a little proportion is transferred to rice grains (Fageria et al., 1982). From the absorbed K, 76 to 86% is found in shoot and 11 to 21% in grains, depending on the cultivar (Fageria, 1991). Potassium content under 4 g kg^{-1} is considered deficient (Reuter and Robinson, 1997). The different doses of rocks promoted

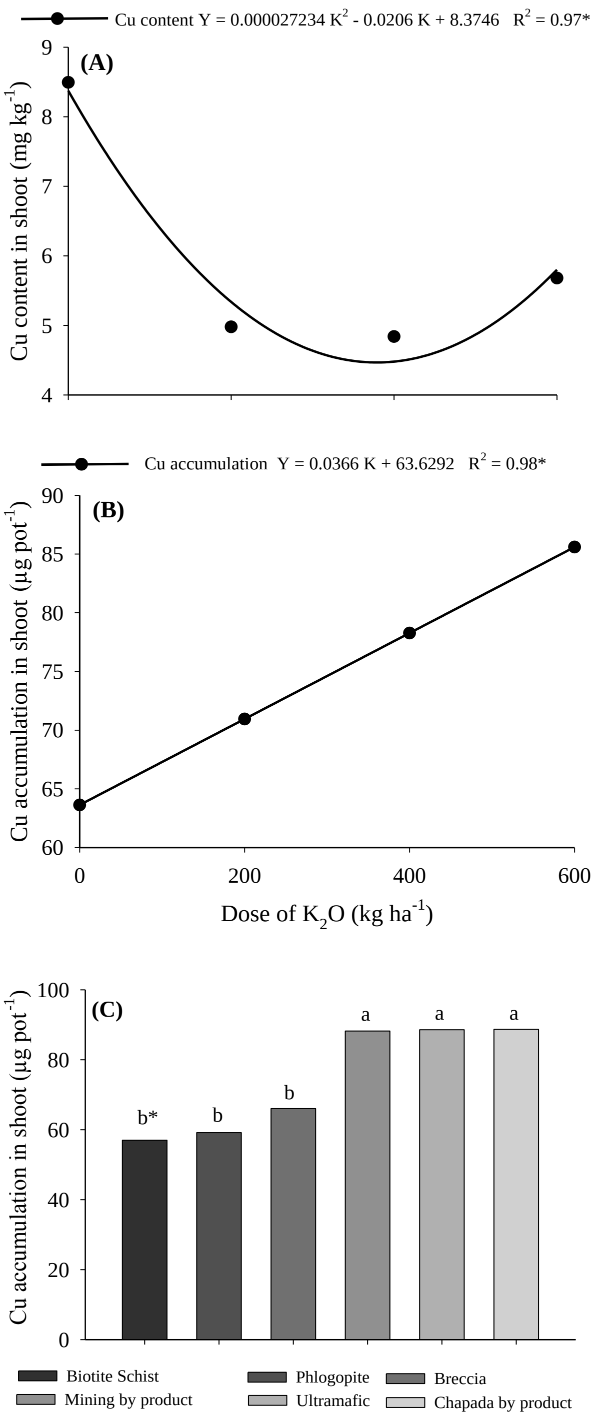

Figure 4. Effect of crushed rocks applied as equivalent K_2O dose on copper content (A) and accumulation (B), (C) in rice shoot. * Numbers followed by same letter are not statistically different by Scott-Knott test (p<0.05).

variable effect also on Ni content and accumulation in rice grains. The decreasing order of Ni accumulation was: breccia > ultramafic > phlogopite = Chapada byproduct > biotite schist > mining byproduct. The average Cu content and accumulation in rice gains, regardless of dose, was high when Chapada byproduct was applied (Figures 4C and 5C). This is directly related to the presence of Cu in the rock (Table 3) with subsequent release to soil, explaining uptake and translocation of this micronutrient.

These high Cu contents, however, never reached the maximum tolerable level of 30 mg kg^{-1} (Abia, 1985). For Zn, the maximum content allowed in grains is 50 mg kg^{-1} and only with mining byproduct this value was reached. When rice was grown in contaminated areas with Cu and Zn, it was observed as plant restricted Cu transport from root to seed but not with Zn (Silva et al., 2007). Crop nutrient use efficiency is important in assessing plants in terms of nutrient use and yield response. Agronomic efficiency and potassium recovery decreased with increasing doses applied as crushed rocks (Figure 7). The decreasing order of rocks for agronomic efficiency was as follows: Ultramafic > Chapada byproduct = mining byproduct > Breccia > phlogopite > Biotite Schist. The first three were considerably higher than the others and promoted considerable plant response on grain yield per kg of K_2O applied. Potassium use efficiency of rice genotypes indicated by agronomic efficiency varies between 4.11 to 11.33 mg mg^{-1} with an average of 8.9 mg mg^{-1} (Fageria et al., 2010). The crushed rocks generated values of agronomic efficiency varying with doses of K_2O and with types of crushed rock, in a range from 2.4 to 22.9 mg mg^{-1}.This remarks the difference among rocks in their capacity to release K from their structures. Consequently, the nutrient release to plants showed different yield response to crushed rock (Table 3) and acidity neutralizer (Table 4). The plant capacity to absorb nutrients from soil solution is closely related to potassium agronomic efficiency. Potassium transport in soil is predominantly through diffusion while mass flow responds for a small proportion (Rosolem et al., 2003). Therefore, the root volume with increased superficial area provides the contact of K from soil solution explaining the results obtained here.

The response in rice, yield measured by potassium agronomic efficiency was directly related to root growth (Figure 1C). The potassium recovery was higher by mining byproduct and ultramafic, reinforcing the feasibility of these minerals as an important source of K. At high doses, mining byproduct did not differ from Chapada byproduct and ultramafic. The latter combine nutrients source with acidity neutralizing power, useful in acid, not limed, areas, common in large part of Brazilian savannah. In absence of K application and with potassium chloride application, recovery rates range from 4.9 to 38.6% respectively for different rice genotypes (Fageria et al., 2010). It becomes evident that crushed rocks may be utilized as nutrients sources due to their content of potassium, nickel, copper and zinc release, as measured by accumulation of these nutrients in rice shoot and grain, with positive effect on yield. Furthermore, these crushed rocks have differentiated acidity neutralization power, impacting positively soil amendments. Rice potassium agronomic efficiency and recovery decreased

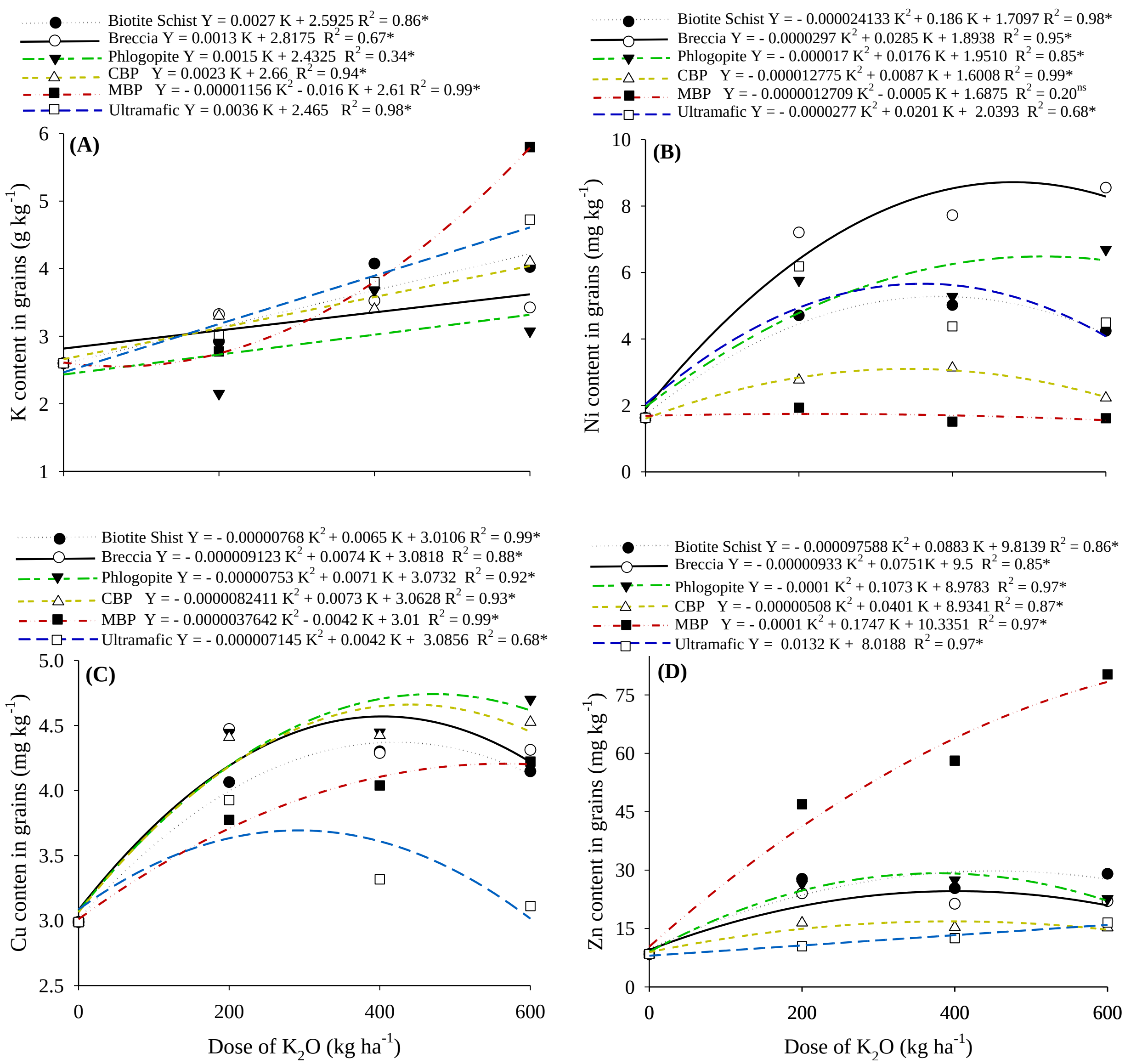

Figure 5. Effect of crushed rocks applied as equivalent K_2O dose on potassium (A), nickel (B), cooper (C) and zinc (D) content in rice grains.

with increasing application of crushed rocks, similar to soluble nutrient sources. Mining byproduct is not only a potassium source but also an important source of Zn with more than 2.8% of this nutrient in its composition.

Ultramafic rock and mining byproduct can be used in upland rice, as measured by yield and potassium use efficiency.

Conclusions

The mining byproducts and potassium-containing-rocks are potential suppliers of micronutrients in addition to reducing acidity in savannah soils. Potassium is highly recovered from mining byproduct and ultramafic. The high doses required to supply K for some material may limit their use in scale farming. Most of them are sources of micronutrients as Zn, Cu, Ni at levels that do not reach toxicity. The slow nutrient release by these materials enables their use in organic farming and in crop-livestock integrated cropping systems. Field experiments are suggested to clarify potential and limitations in the use of these materials as alternative fertilizers.

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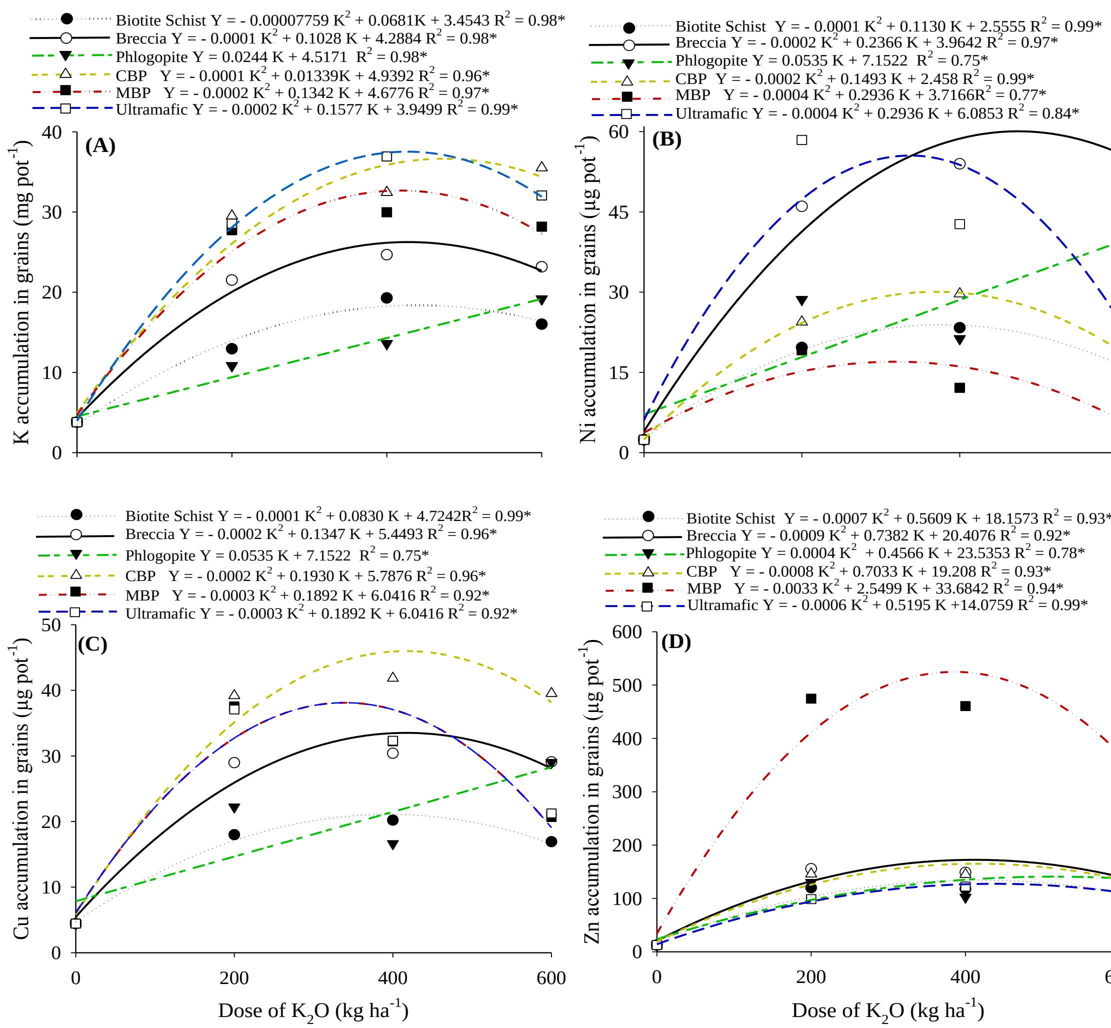

Figure 6. Effect of crushed rocks applied as equivalent K_2O dose on potassium (A), nickel (B), cooper (C) and zinc (D) accumulation in rice grains.

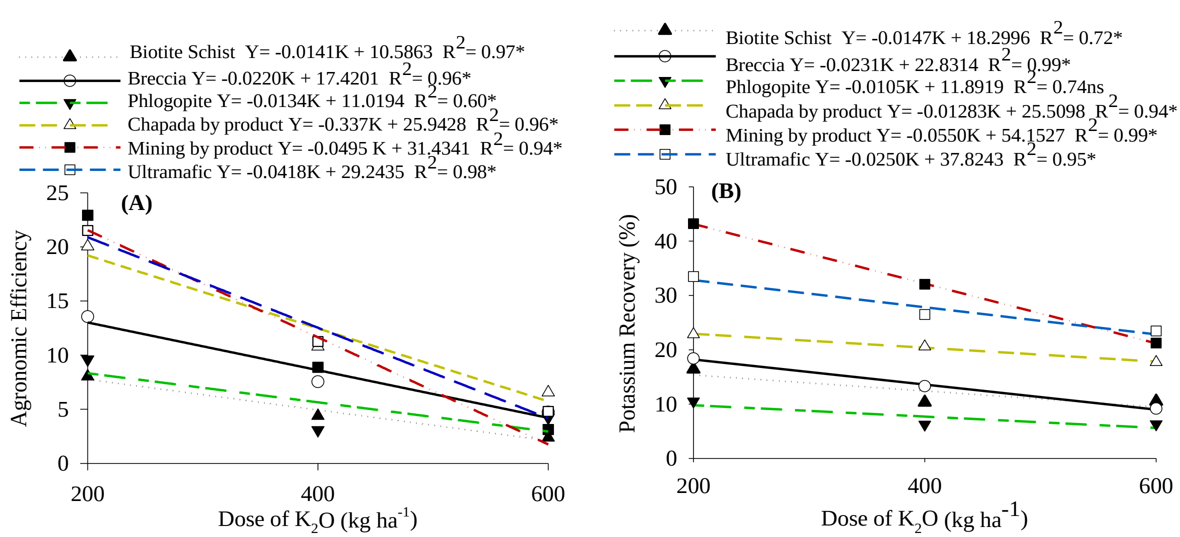

Figure 7. Agronomic efficiency (A) and potassium recovery (B) by rice fertilized with alternative sources of nutrients.

supporting this research.

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Full Length Research Paper

The physical and chemical characteristics of vineyard soils and its heavy metal content in semi-arid environments

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Turkey is one of the most important seedless raisin producers in the world market. Approximately, 82% of the seedless raisin has been produced in Aegean Region of Turkey and 25% of it has been produced in the Plain of Alasehir, located in Aegean Region. Therefore, the Plain of Alasehir was selected to study the physical and chemical characteristics, and the heavy metal contents of the vineyard soils. Hence, the soil samples were collected from 26 different locations in 13 different vineyards to analyze the macro and micro elements, such as total N (TN), P, K, Ca, Mg, Fe, Zn, Mn, and Cu and, the total heavy metal contents, such as Fe, Zn, Mn, Cu, Ni, Co, Pb, Cd, and Cr. Results showed that the soil samples were moderately alkaline. The salinity was suitable to grow seedless grape. The soil textures were either lime or sandy-loam with poor organic matter content. TN, P, Ca, and Mg concentrations were sufficient in the soil; however, 38.5 % of the samples were insufficient for K content. Similarly, Mn and Cu concentrations were sufficient while Fe and Zn were insufficient. Boron concentrations were high in 76.9% of the soil samples, which is a great concern for seedless grape production. Cd and Cr toxicity were not detected while Pb pollution was observed in only one vineyard. There was Co pollution in the 23.1% and %53.8 of the soil samples for the first and the second depth, respectively. Ni pollution was detected in some soil samples.

Key words: Vineyards (*Vitis vinifera* L.), macro elements, micro element, soil productivity, heavy metal, raisin.

INTRODUCTION

Substances or energies introduced into the environment that has undesired effects, or adversely effect of usefulness of a resource is called "pollutants". In general, pollutants are originated from domestic, industrial, and agricultural practices. One of the most harmful pollutants is the heavy metal based chemicals. Analysis of heavy metals in environmental samples caught the attention of researchers in the recent years, since they have toxic effects on living organisms. Removing the heavy metals from the soil is very hard and an expensive process.

The contamination level in soil has increased with industrial developments because of waste disposal from industrial facilities to environment including metals, heavy metal salts and some other hazardous chemicals. Heavy

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metals are microelements (trace elements) needed by animals and plants; however, those microelements can become toxic when they exist above certain limits (Alloway, 1990). Depending on the type of facilities, industrial wastes may contain Fe, Zn, Cu, Mn, B, Pb, Cd, Ni, Cr, and Co, which may cause contamination when accumulated in the soil. Fertilizers, pesticides, and hormones used in agricultural applications are the other sources of contaminants for soils (Forstner et al., 1991; Jaraush-Wehrheim et al., 2001; Elmaci et al., 2002). Thus, a complex movement of elements in the environment needs to be monitored continuously. Also, the uptake of heavy metals by plants depends on factors such as plant variety, species, plant part, age of organ or plant, growth period, stability to heavy metals, and transfer factor of heavy metals (Fergusson, 1990; Secer et al., 2002).

Among the other countries, Turkey is one of the leading countries for high quality seedless raisin production and exportation since western part of Turkey has semi-arid climate, which is suitable for seedless grape growing. Turkey has large amount of vineyard farms that produce a wide variety and high volume of seedless grape. Most of the grapes produced in the region are processed to produce raisin. About 82% of Turkey's seedless raisin is produced in the Gediz Basin, which is in the Aegean Region. The majority of seedless grape is grown in the vicinity of several cities in the region, such as Izmir, Denizli, and Manisa. Particularly, the Plain of Alasehir in Manisa area covers about 25% of, seedless raisin production of the entire Aegean Region. About 90% of the total production of vineyards in the Plain of Alasehir is the round type of seedless raisin (*Vitis vinifera* L.) (Aydin et al., 2007).

Soil, water, and air pollution which is defined as environmental pollution leads to contamination in agricultural areas and also have negative impact on the quality and quantity of plant production. Moreover, this issue has threatening effects on human health as part of their food chain. Wrong agricultural practices in the region to increase the size, endurance, and quality of grapes have caused undesirable conditions in some of the vineyard soils. This issue also had negative impact on foreign market over the years.

Considering the Plain of Alasehir's seedless raisin production potential, maintaining the high quality of soil in vineyards gained attention in the region to increase the quality and the volume of raisin production. Therefore, the soil samples were collected from 26 different locations in 13 different vineyards to analyze the macro and micro elements, such as total N (TN), P, K, Ca, Mg, Fe, Zn, Mn, and Cu and the total heavy metal contents, such as Fe, Zn, Mn, Cu, Ni, Co, Pb, Cd, and Cr.

MATERIALS AND METHODS

In this study, 26 soil samples were collected from 13 vineyards (*Vitis vinifera* L.) during the verasion period in August 2009 from the Plain of Alasehir in Aegean Region, Turkey. Locations of the samples are presented in Table 1. The soil samples were collected from two different depths, which were 0 to 30 cm and 30 to 60 cm to analyze the total heavy metal contents, which were Fe, Zn, Mn, Cu, Ni, Co, Pb, Cd, and Cr and some of the macro and micro elements, which were total N, available P, K, Ca, Mg, Fe, Zn, Mn, and Cu. According to the new soil taxonomy system, the type of soils in this study can be classified as typical xerofluent soils (Altinbas et al., 1994).

The soil samples were prepared according to Anonymous (1951), pH analysis was made according to Jackson (1967), lime analyzed according to Schlichting and Blume (1966), total soluble salt to Anonymous (1951), soil structure to Bouyoucos (1962), the amount of organic C that was determined by method of wet burning organic matter by multiplying 1.724 factor to Reuterberg and Kremkus (1951), total N, with the modified macro Kjeldahl method to Bremner (1965), available P to Bingham (1949) and it was classified to Guner (1968). Available Fe, Zn, Mn and Cu, following the methods of DTPA-TEA and using an Atomic Absorbsion Spectrophotometre to Lindsay and Norvell (1978), available B was determined using colorimetric method to Riehm (1957). Furthermore, the total N content of the soils was analyzed using a modified Kjeldal method according to Bremner (1965) and the soil classification was done according to Kovanci (1985). Available K, Ca and Mg, were extracted in 1N NH_4OAC and K and Ca concentrations were determined using flame spectrophotometric method while Mg was determined using an atomic absorption spectrometer (AAS) (Pratt, 1965) and classification was done according to the Loue (1968). Total heavy metal and micro element contents (Cd, Co, Cr, Ni, Pb, Fe, Zn, Mn, Cu) were extracted using the aqua regia extraction method prior to analyzing using an AAS method (Slawin, 1968; Kick et al., 1980).

RESULTS AND DISCUSSION

Some physical characteristics, total N and available macro and micro element contents of soils

The soil samples were collected from the vineyards in the Plain of Alasehir in Aegean Region. The results of total soluble salt in water, pH, $CaCO_3$, texture, percentages of sand, silt, clay, and organic matter are presented in Table 2. Total N and available macro and micro element contents (P, K, Ca, Mg, Fe, Zn, Mn, Cu, and B) were presented in Table 3. When pH results were classified according to Kelogg (1952), 76.9% of soil samples collected from 0 to 30 cm depth were light alkaline (pH of 7.4-7.8) and 23.1% of the soil samples were medium alkaline (pH of 7.9-8.4). For the samples collected from 30 to 60 cm depth, 69.2% of the samples were light alkaline and 30.8% were medium alkaline. Therefore, the majority of the soil samples, which displayed light alkaline properties, were suitable for viticulture. In terms of total soluble salt content in the soil samples collected from both depths, about 7.7% of the samples were determined as light salty (0.15 to 0.35%) while about 92.3% of the soil sample were determined as very low salty (>0.15%), which was not toxic to the grape production (Anonymous, 1951).

Results for the samples collected from the depth of 0 to lime soil (2.5 to 5.0% lime), 30.8% of the samples were

Table 1. Soil samples locations.

Sample no.	Vineyard location	Name of the owner
1	Kovalık	Mehmet Ok
2	Mezarlık	Mustafa Onal
3	Saglık ocagı	Mehmet Aktaş
4	Kovalık	Ahmet Yildirim
5	Sellik	Suleyman Ceylan
6	Plain of Alasehir	Cevdet Yavuz
7	Plain of Alasehir	Huseyin Basturk
8	Plain of Alasehir	Suleyman Basturk
9	Plain of Alasehir	Faruk Ozturk
10	Plain of Alasehir	Cengiz Ozturk
11	Plain of Alasehir	Izzet Cetin
12	Plain of Alasehir	Huseyin Topaloglu
13	Plain of Alasehir	Cengiz Sahin

texture + lime (20.0 to 50.0% texture + lime), and 7.7% of the samples were rich based on lime (5.0 to 10.0% lime). For the samples collected from the depth of 30 to 60 cm, 53.8% of the samples were lime, 23.1% of the samples were texture + lime, 15.4% of the samples were texture + marl (10.0 to 20.0% texture + marl), and 7.7% of the samples were poor based on lime (Evliya, 1960). Furthermore, about 76.9% of soil samples collected from both depths were sandy-loam, 15.4% of the samples were loam, and 7.7% of the samples were silt-loam texture (2.5% silt-load texture) (Table 2). Similarly, Celik (1998) reported that the sandy-loam texture is necessary for viticulture. The organic matter content of the soil samples were as follows: for the samples collected from 0 to 30 cm depth; 61.5% of the samples were poor in terms of humic (>2%), 38.5% of samples were consider as humic (2 to 4%), for the samples collected from 30 to 60 cm depth; 92.3% of the samples were poor in terms of humic except the sample collected location 11 was humic according to classification by Schaffer and Schachtschabel (1967). These results explained that organic matter content in second depth was lower than that of first depth (Table 2).

Total nitrogen (N) content of the soil samples collected from both depths were measured and results based on nitrogen content expressed that: for the first depth; 61.5% of soil samples were medium (0.05 to 0.10%), 30.8% of samples were suitable (0.10 to 0.15%), and 7.7% of samples were rich (>0.15%); for the second depth, 84.6% of samples were medium, 7.7% of samples were rich, and 7.7% of the samples, which was location 1, was poor (Kovanci, 1985). These results showed that the vineyard soils from both depths were either medium or rich in terms of nitrogen content (Table 3).

The results from phosphorous analyses, which were examined according to the criteria values given by Guner(1968), for the both depths showed that the available phosphorus content was suitable for grape production(>3.26 mg.kg^{-1}) in all the locations. In addition, available potassium (K) for the first depth reported as follows: 30.8% of the soil samples were rich (300 to 400 mg.kg^{-1}), 23.1% of the soil samples were suitable (200 to 300 mg.kg^{-1}), 23.1% of the soil samples were inadequate (<150 mg.kg^{-1}), 15.4% of the soil samples were poor (150 to 200 mg.kg^{-1}), and 7.7% of the soil samples were excessive (<400 mg.kg^{-1}), and available potassium for the second depth explained as; 38.5% of the soil samples were inadequate, 30.8% of the soil samples were adequate, 15.4% of soil samples were rich, and 15.4% of the soil samples were poor based on the classification by Fawzi and El-Fouly (1980). Results showed that available potassium contents in the soil samples collected in the first depth were higher than that of the second depth and Altinbas et al. (1994) and Yener et al. (2002) was also observed in the same situation for the samples collected from the vineyards in the same area.

Calcium (Ca) contents of the soils in both depths were analyzed according to the criteria values presented by Loue (1968). In terms of Ca content of the soil samples, the results can be summarized as follows: for the samples collected from 0 to 30 cm depth, 15.4% of the samples were excessive (>3571 mg.kg^{-1}), 46.2% of the samples were rich (2857 to 3571 mg.kg^{-1}), and 38.5% of the samples were in suitable level (2143 to 2857 mg.kg^{-1}); for the samples collected from 30-60 cm depth; 7.7% of the samples were excessive, 53.8% of the samples were rich, and 38.5% of the samples were in suitable level. Results proved that the amount of Ca in both depths were adequate and very close each other. Magnesium (Mg) contents of the soil samples were also analyzed. Results showed that 92.3% of the soil samples in both depths was very excessive in terms of Mg (>350 mg.kg^{-1}) except the sample in location 5 in second depth had slightly lower Mg compare to the Mg content in other locations according to Loue (1968). Literatures expressed that the magnesium level in the majority of vineyard soils

Table 2. Physical and chemical properties of soil samples collected from the Plain of Alasehir vineyards.

Sample no	Sampling depth (cm)	pH	Total soluble salt (%)	Soil type				Soil texture	Organic matter (%)
				Lime (%)	Sand (%)	Silt (%)	Clay (%)		
1	0-30	7.98	0.031	23.78	54.24	36.00	9.76	Sandy loam	1.81
	30-60	8.03	<0.030	21.37	60.24	34.00	5.76	Sandy loam	1.05
2	0-30	7.81	0.045	26.62	62.24	26.00	11.76	Sandy loam	1.77
	30-60	7.85	0.038	24.42	62.24	30.00	7.76	Sandy loam	1.58
3	0-30	7.72	0.048	22.51	60.24	28.00	11.76	Sandy loam	1.74
	30-60	7.76	0.032	19.59	54.24	30.00	15.76	Sandy loam	1.63
4	0-30	7.79	0.045	33.22	62.24	20.00	17.76	Sandy loam	2.10
	30-60	7.75	0.050	34.21	68.24	16.00	15.76	Sandy loam	1.93
5	0-30	7.98	<0.030	9.69	76.24	16.00	7.76	Sandy loam	1.29
	30-60	8.04	<0.030	11.57	68.24	26.00	5.76	Sandy loam	1.16
6	0-30	7.70	0.062	3.04	72.24	22.00	5.76	Sandy loam	1.93
	30-60	7.96	0.055	2.01	70.24	22.00	7.76	Sandy loam	1.06
7	0-30	7.93	0.048	3.59	62.24	28.00	9.76	Sandy loam	1.56
	30-60	7.98	0.060	3.55	62.24	32.00	5.76	Sandy loam	1.46
8	0-30	7.62	0.280	4.66	52.24	36.00	11.76	Loam	2.21
	30-60	7.74	0.330	4.70	44.24	44.00	11.76	Loam	1.91
9	0-30	7.56	0.085	4.18	34.24	58.00	7.76	Silt loam	2.81
	30-60	7.74	0.070	3.63	38.24	52.00	9.76	Silt loam	1.79
10	0-30	7.60	0.061	3.71	46.24	44.00	9.76	Loamy	2.66
	30-60	7.65	0.063	3.77	50.24	38.00	11.76	Loamy	1.54
11	0-30	7.51	0.098	2.84	56.24	38.00	5.76	Sandy loam	2.86
	30-60	7.67	0.096	2.53	60.24	34.00	5.76	Sandy loam	2.34
12	0-30	7.74	0.045	4.54	60.24	26.00	13.76	Sandy loam	1.55
	30-60	7.84	0.046	4.50	62.24	28.00	9.76	Sandy loam	1.39
13	0-30	7.55	0.140	3.16	54.24	40.00	5.76	Sandy loam	1.98
	30-60	7.70	0.170	3.19	58.24	34.00	7.76	Sandy loam	1.69
Average		7.78	0.080	10.95	58.16	32.23	9.61	-	1.80

in various regions of Turkey was sufficient (Danisman et al., 1983; Altinbas et al., 1994; Yener et al., 2002; Elmaci et al., 2002).

Microelements were analyzed in all the soil samples collected from both depths in the Plain of Alasehir vineyards (Table 3). Results showed that 15.4% of the soil samples collected from both depth was insufficient based on Fe level (2.5 to 4.5 mg.kg^{-1}) while 84.6% of the samples were sufficient (>4.5 mg.kg^{-1}). Zn results for the soil samples collected from the first depth showed that 76.9% of the samples were sufficient (>1 mg.kg^{-1}) and 23.1% of the samples were in critical level (0.5 to 1 ppm) while in second depth; 53.8% of the samples were sufficient, 30.8% of the samples were in critical level, and 15.4% of the samples were insufficient (>0.5 mg.kg^{-1}) based on the values given by Lindsay and Norwell (1978). High lime ($CaCO_3$) content with lack of available Fe and Zn was also observed in the soils as shown in Table 3.

Cu and Mn contents of all of the soils samples from both depths were in an adequate level (1 to 2 mg.kg^{-1} available Mn; >0 to 2 mg.kg^{-1} available Cu) according to Lindsay and Norwell (1978). Cu content of the samples were higher in 0 to 30 cm depth compare to the Cu content in 30 to 60 cm depth. Available boron (B) content was determined in both depths and 76.9% of the samples were found in toxic level for the vineyard plants (0.5 to 2.0 ppm) (Scheffer and Schacthschabel, 1989). Previous studies also found similar results for boron content in the vineyard soils in Kavaklidere, Alasehir area, which is a

Table 3. Total N and available macro and micro element contents of the soil samples collected from the vineyard plantations.

Sample no	Depth (cm)	Total N (%)	Available ($mg.kg^{-1}$)								
			P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
1	0-30	0.084	15.4	410	3775	480	3.12	2.93	0.70	14.4	0.20
	30-60	0.053	5.2	295	3345	520	2.86	2.23	0.64	9.8	0.24
2	0-30	0.095	9.9	125	3660	420	5.32	2.42	1.52	27.1	0.14
	30-60	0.078	9.1	85	3545	500	4.75	2.26	0.58	15.3	0.18
3	0-30	0.095	11.4	285	3490	480	5.63	2.02	0.88	14.6	0.16
	30-60	0.081	7.5	220	3490	440	5.54	1.20	0.56	11.3	0.16
4	0-30	0.101	7.5	370	3490	420	5.10	2.06	1.56	14.6	1.27
	30-60	0.079	7.4	360	3605	460	4.09	1.85	1.40	14.0	1.32
5	0-30	0.064	9.6	95	2860	360	3.08	3.94	0.54	13.4	1.50
	30-60	0.056	5.4	85	3035	300	2.77	1.37	0.46	12.2	1.75
6	0-30	0.095	9.6	115	2405	440	29.0	2.52	1.02	22.4	1.11
	30-60	0.053	3.9	95	2230	360	16.0	1.10	0.48	15.7	1.01
7	0-30	0.081	15.0	165	2860	580	24.2	2.26	1.32	17.3	1.04
	30-60	0.071	6.7	115	2750	580	18.9	1.49	0.86	12.4	1.05
8	0-30	0.115	18.1	390	2575	560	53.7	3.48	2.60	16.4	1.00
	30-60	0.081	13.7	155	3050	740	26.0	2.21	1.60	16.2	0.96
9	0-30	0.134	22.7	285	3100	440	48.0	3.72	1.88	14.7	1.08
	30-60	0.092	18.1	240	2700	500	35.2	3.17	1.30	18.2	1.16
10	0-30	0.134	20.5	230	2745	440	62.9	5.18	3.40	23.1	1.04
	30-60	0.062	10.9	155	2945	360	40.9	2.59	2.80	31.7	1.10
11	0-30	0.157	20.1	305	2745	400	70.8	6.86	3.20	22.4	1.22
	30-60	0.112	15.6	295	2405	280	31.2	2.47	1.46	12.4	1.14
12	0-30	0.084	14.8	190	2945	320	40.0	3.62	1.94	22.8	1.12
	30-60	0.076	11.8	135	2805	480	38.7	2.69	1.70	24.4	1.10
13	0-30	0.109	16.4	400	2700	460	26.0	2.74	1.90	17.8	1.18
	30-60	0.081	16.1	315	2750	380	27.1	2.04	1.16	12.9	1.25
Average		0.090	12.4	228	3,000	450	24.3	2.71	1.44	17.2	0.94

close area for the sample locations used in this study (Konuk and Yener, 1995; Yener et al., 2002). Boron content in irrigation waters was also found high in this area. One possible reason for high boron content in the irrigation water can be explained that the hot spring water resources located in the area cause increase in the boron content in the groundwater. Thus; we can state that boron is possible to become a problem in vineyards in the Plain of Alasehir in the near future.

Total micro elements and heavy metal contents of soil that is soluble in Aqua Regia

Minimum, maximum and average values of total micro elements and heavy metal (Fe, Zn, Cu, Cd, Co, Ni, Pb) contents of soil samples collected from vineyards plantations in both depths (0 to 30 cm and 30 to 60 cm) are presented in Table 4. According to Scheffer and Schachtschabel (1989), the limit values of Fe in soils in both depths were 0.5 to 5.0% of Fe, which were not toxic for vineyard soils. In terms of total Zn content, there was no Zn pollution risk in both depths in sampling locations since Zn values analyzed in this study were lower than the limit value of 300 $mg.kg^{-1}$ given by Kloke (1980) for vineyard soils. Total Mn content in both depths were also under the limit values (1500 to 3000 $mg.kg^{-1}$) given by Pendias and Pendias (1992) for vineyard soils. Besides, it was noticeable that the amount of Mn in the samples found as well below the critical Mn limits. Furthermore, results from this study showed that the total Cu content which was extracted using the aqua regia ($3HCl+HNO_3$)

Table 4. Total micro elements and heavy metal contents of the soil samples collected from the vineyard plantations.

Sample no	Depth (cm)	Total Fe (%)	Total ($mg.kg^{-1}$)							
			Cu	Zn	Mn	Co	Pb	Cr	Cd	Ni
1	0-30	1.89	38.5	112.4	495.0	28.0	69.3	28.2	2.26	43.8
	30-60	1.91	34.8	65.9	522.5	25.4	48.9	27.3	1.93	41.0
2	0-30	1.89	33.6	73.6	481.3	27.1	124.7	23.0	2.04	36.6
	30-60	1.78	35.8	62.0	495.0	28.0	185.4	25.3	2.04	38.0
3	0-30	1.86	24.9	62.0	357.5	22.3	40.3	25.0	1.82	37.2
	30-60	1.65	22.4	46.5	275.0	23.6	41.4	23.9	1.93	34.6
4	0-30	1.65	23.6	81.4	440.0	25.4	34.9	27.6	2.37	37.6
	30-60	1.54	22.9	73.6	288.8	25.8	34.9	26.2	2.36	38.3
5	0-30	1.69	34.9	62.0	316.2	20.6	22.6	23.9	1.21	33.4
	30-60	2.29	27.5	58.1	343.7	21.9	29.6	24.4	1.27	37.2
6	0-30	1.89	3.01	73.6	398.8	20.6	17.2	31.6	0.66	45.3
	30-60	2.08	23.6	100.7	343.8	18.4	9.7	27.9	0.61	34.8
7	0-30	2.81	32.7	143.3	426.3	22.3	16.7	35.1	0.72	47.2
	30-60	2.53	29.6	93.0	398.8	21.9	15.6	26.0	1.05	41.2
8	0-30	2.88	34.9	108.5	440.0	22.8	18.3	35.1	0.66	53.8
	30-60	2.96	39.7	155.0	495.0	25.8	19.9	41.7	0.88	50.4
9	0-30	2.67	30.1	174.4	343.7	23.1	14.5	34.2	0.83	34.4
	30-60	3.28	37.1	162.7	440.0	25.8	18.3	42.8	0.99	52.7
10	0-30	2.91	39.3	104.6	440.0	24.1	16.7	37.7	0.88	48.4
	30-60	3.02	35.2	132.6	360.0	25.6	17.2	39.4	0.96	56.5
11	0-30	2.12	45.2	93.0	412.5	21.0	19.9	36.8	0.88	44.8
	30-60	2.79	34.5	96.9	398.0	25.4	19.4	37.1	0.77	46.3
12	0-30	2.72	39.5	135.6	522.5	23.2	18.8	35.4	0.83	55.5
	30-60	2.72	37.9	166.7	495.0	24.1	18.8	34.2	0.77	56.1
13	0-30	3.00	35.1	135.6	440.0	24.5	15.6	38.5	1.05	45.7
	30-60	2.83	30.7	155.0	398.8	21.9	14.5	26.5	0.66	40.8
Average		2.36	31.8	105.0	414.2	23.8	34.7	31.3	1.25	43.5

was well below the standard levels, which were explained as 100 $mg.kg^{-1}$ by Kloke (1980) and 60 to 125 $mg.kg^{-1}$ by Pendias and Pendias (1992) (Table 4).

Total Cu contents of the soils samples generally decreased from first depth to the second depth. Hakerlerler et al. (1994), Elmaci et al. (2002) and Tuna et al. (2005), studied heavy metal pollution in the soil samples in the same area as in this study and they found the similar results as in the case of this study for Fe, Zn, Mn, and Cu. Additionally, Cd was analyzed in the soil samples and a high value of Cd was determined in especially vineyards that were close to industrial areas where exhaust gases and chemical fertilizers (superphosphate) were observed distinctively. In general, Cd content in most of the vineyards was under the well below the standard levels (3 $mg.kg^{-1}$) presented by Kloke (1980) and Pendias and Pendias (1992). According to these criteria, it was determined that there was no Cd toxicity in vineyard soils in the Plain of Alaşehir. Moreover, Alloway (1990) and Altinbas et al. (1994) reported that Cd was more concentrated in upper soil layers and its mobility was more than that of Pb and Cu along the profile while it was shown a similar mobility with Ni and Zn. In terms of total Co; there was no Co pollution according to the critical value (50 $mg.kg^{-1}$) given by Kloke (1980) in soils from both depths. According to the critical values (25 to 50 $mg.kg^{-1}$) given by Pendias and Pendias (1992), Co pollution was determined as 23.1% of soils in the first depth and 53.8% of the soils in the second depth were contaminated with Co (Table 4).

Previous studies have shown that most of the soils in Germany contained 5 to 100 ppm Cr even though in some cases the concentrations went up to 300 ppm (Scheffer and Shachtschabel, 1989). Based on the values of 75 to 100 ppm Cr content given by Pendias and Pendias (1992) and 100 ppm Cr pollution given by Kloke (1980), there was no pollution concern in the region for this metal. While Pendias and Pendias (1992) reported the critical value for Ni in soils as 100 ppm, Kloke (1980) reported the critical value as 50 ppm for this metal. If we consider the 50 ppm Ni concentration as a critical value as reported by Kloke (1980), Ni pollution was determined in the 15.4% of soils at the first depth, and in 30.8% of soils at the second depth (Table 4). The amount of the Ni in the collected soil samples from both depths was close to the limit value (50 ppm), (Table 4). The total Ni in the agricultural soils was reported between 28.7 and 86.2 ppm from the Gediz region (Altinbas et al., 1994) and between 22.3 and 962.5 ppm in the Manisa and Menemen plain (Elmaci et al., 2002). Additional to agricultural and industrial practices that may cause pollution in the soils, the distance between agricultural areas and the highways are linked to the soil pollution in terms of Cd, Pb and Ni concentrations (Ndiokwere, 1984; Hakerlerler et al., 1994). Pb was detected between 1 and 20 ppm in slightly polluted soils and between 25 and 95 ppm in more polluted soils (Scheffer and Schachtschabel, 1989). Kloke (1980) reported that the critical value of Pb pollution was 100 ppm in soils. According to this critical value by Kloke (1980), there is a Pb pollution in 92.3% of the soils in both depths, except vineyard number 2. The source of Pb pollution can be the use of phosphorus fertilizer, Pb containing fuel and Pb transmitted with air movements.

Conclusion

The soil samples, which were collected from the Plain of Alasehir vineyards, were analyzed and results showed that; the alkalinity of the soils was ranged between light and medium and the salinity was at optimum level, which was not harmful for the grape production. The lime and sandy-loam texture were determined with poor organic matter content. All the soil samples were sufficient in terms of total N and available P. Furthermore, available Ca and Mg content in all the soil samples and K content in 61.5% of the soil samples were at sufficient level. Light and medium alkalinities with rich lime content in the soils indicate possible phosphorus fixation issue. In this regard it has been recommended that phosphorus fertilization could be applied to the band and 20 to 30 cm deep in vineyards located in the Plain of Alasehir. In terms of the micro element contents; while the Fe and Zn content level were not sufficient, in all of the vineyards available Mn and Cu contents were at sufficient level. Available boron content was found in high level which could create a toxicity problem in 76.9% of the soils. One possible source for high boron content in the soils can be explained as high boron content in the groundwater sources in the vineyard area. Therefore, the quality of irrigation water should be monitored continuously in the region.

The total micro element contents of the soil samples were examined in the aqua regia and results showed that there was no pollution in terms of total Fe, Zn, Mn and Cu contents. The heavy metal contents were also determined and found no Cd and Cr toxicities in the soils. It was also determined that there was no Pb pollution of 93.2% of the soil samples except the samples collected from vineyard number 2. Co pollution was determined in 23.1% of the soil samples in the first depth while 53.8% of the samples in the second depth. Similarly, Ni pollution was determined by 15.4% of the soil samples in the first depth while 30.8% of the samples in the second depth. While Co pollution might be caused by industrial facilities, Ni and Pb pollutions might be caused from exhaust gases from the vehicles in the area.

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Full Length Research Paper

Evaluation of the laser leveled land leveling technology on crop yield and water use productivity in Western Uttar Pradesh

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A study has been conducted for 3 year on impacts of the laser land leveling versus traditional land leveling on water use productivity and crop yields. The major concerns were effectiveness of laser land leveling as a water saving tool in the new context of land use and ownership, affordability of laser land leveling for farmers and the economic viability of this technology. These research questions were studied in a sizable area of laser leveled and neighboring non-leveled (control) fields for 2009 to 2011. The result indicated that with laser leveling, farmers could save irrigation water 21%, energy by 31% and obtained 6.6, 5.4 and 10.9% in rice, wheat and sugarcane higher yields. The total irrigation duration and applied water depth was reduced to 10.9, 14.7% in rice; 13.7, 13.3% in wheat and 13.5, 20.3% in sugar-cane as compared to traditional leveled fields. The laser leveled fields exhibited the highest water use efficiency (WUE), which was 48, 47 and 49% higher in precisely leveled field than control (unleveled), 22, 19 and 20% higher than traditionally leveling fields, respectively. The average water productivity in rice, wheat and sugarcane has improved by 33%. The average annual net income from the laser field was 14, 13.5 and 23.8% in rice, wheat, sugarcane higher than that from the traditional leveled field. It was concluded that the use of laser land leveling increases yield and saves irrigation water as compared to traditional method of leveling in different cropping system prevailing in western U.P.

Key words: Crop productivity, laser leveled land leveling, water use efficiency, water productivity.

INTRODUCTION

Declining water table and degrading soil health are the major concerns for the current growth rate and sustainability of Indian Agriculture. Thus, proper emphasis is being given on the management of irrigation water usage for adequate growth of agriculture. Keeping in view, the need for judicious use of our natural resources, concerted efforts are being made to enlighten the farmers for efficient use of irrigation water at farm level (Kaur et al., 2012). Generally, in sugarcane-wheat and rice-wheat, rotation farmers believed that their fields are leveled and needed no further leveling. But the digital elevation survey sheet of a field shows that most of the

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fields are not adequately leveled and requires further precision land leveling. The enhancement of water use efficiency and farm productivity at field level is one of the best options to readdress the problem of declining water level in the state. The planner and policy makers are properly informed and motivated to develop strategies and programs for efficient utilization of available water resources. Laser Land leveling is one such important technology for using water efficiently as it reduces irrigation time and enhances productivity not only of water but also of other non-water farm inputs.

The use of laser technology in the precision land leveling is of recent origin in India. It does not only minimize the cost of leveling but also ensures the desired degree of precision. However, the laser land leveling was introduced in the state in 2001 by Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U. P.) in collaboration with Rice-Wheat Consortium, New Delhi under the leadership of Dr. R. K. Naresh. Land leveling of farmer's field is an important process in the preparation of land. It enables efficient utilization of scarce water resources through elimination of unnecessary depression and elevated contours (Naresh et al., 2011). It has been noted that poor farm design and uneven fields are responsible for 30% water losses (Asif et al., 2003). Precision land leveling (PLL) facilitated application efficiency through even distribution of water and increased water-use efficiency that resulted in uniform seed germination, better crop growth and higher crop yield (Jat et al., 2006). The scarcity of canal water supplies coupled with unfit ground water has compelled the farmers to utilize available water resources more wisely and efficiently. Under these circumstances, PLL can help the farmers to utilize the scarce land and water resource more effectively and efficiently towards increased crop production (Abdullaev et al., 2007). It was estimated that around 25 to 30% of irrigation water could be saved through this technique without having any adverse affect on the crop yield (Bhatt and Sharma, 2009).

The land leveling have resulted smoother soil surface, reduction in time and water required to irrigate the field, more uniform distribution of water in the field, more uniform moisture environment for crops, more uniform germination and growth of crops, reduction in seed weight, fertilizer, chemicals and fuel used in cultivation, and improved field traffic ability (for subsequent operations). Limitations of laser leveling include high cost of the equipment/laser instrument, the need for a skilled operator to set/adjust laser settings and operate the tractor, and restriction to regularly shaped fields. Farmers, as entrepreneurs are unwilling to adopt new technologies unless they clearly see quick and tangible results in terms of farm profitability. Theoretically, a farmer would opt for a new technology if assurance of earning a net profit were shown. Some economists believe that the net returns must be at least 30% higher than for the traditional technology before farmers would consider adoption. According to an estimate, the number of laser levelers in western Uttar Pradesh has increased sharply from mere 01 in the year 2001 to 350 in the year 2011 of this, on farm resource conservation technologies in States like Uttar Pradesh have an edge over other technologies. Land leveling through laser leveler is one such proven technology that is highly useful in conservation of irrigation water and enhancing productivity. Keeping this in view, this study was undertaken with the objective to access the impact of laser land leveling on the productivity of rice, wheat and sugarcane crop by comparing it with the conventional practice and to find out the extent of water and energy saving as a result of laser or precision land leveling.

MATERIALS AND METHODS

Biophysical, demographic, and socioeconomic profile

Initially, a baseline survey of randomly selected farmers from different villages was conducted to understand their social, economic, and educational status in addition to input use (seed, irrigation, tractor, labor, fertilizer, and pesticide use) and outputs (grain and straw yield) in conventional farmers' practices. The study was conducted for three years from June 2009 to May 2011 in 50 farmers' fields at Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut and Ghaziabad sites. Out of 50 farmers, 57% had land holdings of <2 ha, 31% had 2 to 4 ha, and 12% had more than 4 ha (Figure 1A). About 5% of the farmers were literate, out of which 27% were middle-school pass, 47% were high-school pass, and 21% were college pass (Figure 1B).

The literacy rate was higher for large farmers than for small farmers. The average family size was 6.4 family members. The large farmers usually lived in joint families; whereas medium and small farmers had a separate nuclear family. Out of 320 family members of the 50 households surveyed, 29% were fully engaged in agriculture and 41% were partly engaged, whereas 30% were students who also helped with agricultural activities during vacation and/or leisure periods (Figure 1C). 38% of the farmers were members of different cooperatives existing in the area. Sugarcane and wheat were the major source of income for 52% of the farmers, followed by rice (34%), vegetables (12%), and oilseeds (9%).

Socio economics and demographics of project sites of Uttar Pradesh, India

An experiment was conducted on sugarcane-wheat and rice-wheat rotation in two districts (Meerut and Ghaziabad) in farmers participatory mode in the juridiction of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (Uttar Pradesh), India, (28°40′07″N to 29°28′11″N, 77°28′14″E to 77°4418″E) during 2009 to 2011. The experiment was farmer-managed, with a single replicate, repeated over many farmers. Therefore, the experimental design was Randomized Block Design in which the number of treatments varied from farmer to farmer, with the farmer as a replicate/block. The treatments consisted of Laser land leveling (T_1), Traditional land leveling (T_2) and Control (Unleveled) (T_3).

In treatments T_1 and T_2 leveling of experimental field was done as per treatment and information on the topography of each experimental unit was compiled. The climate of the area is semiarid, with an average annual rainfall of 805 mm (75 to 80% of which is received during July to September), minimum temperature of 4°C in January, maximum temperature of 41 to 45°C in June, and relative

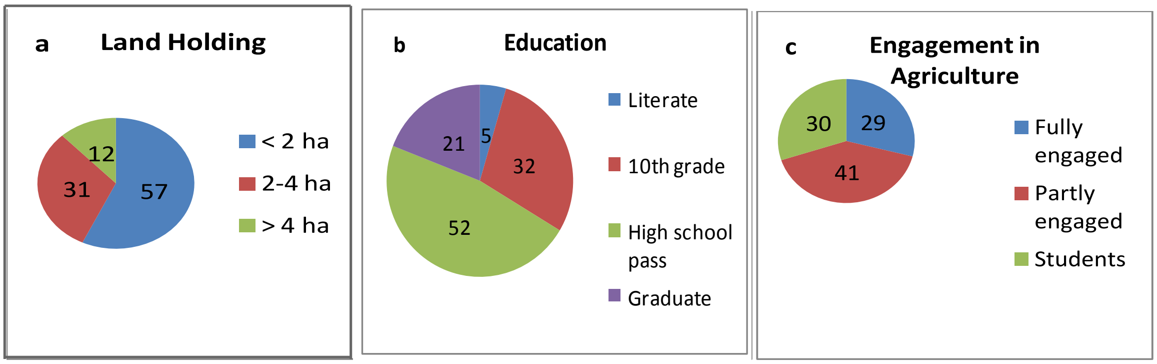

Figure 1. Socioeconomics and demographics of project sites of western Uttar Pradesh, India.

humidity of 67 to 83% during the year. The soils are generally sandy loam to loam in texture and low to medium in organic matter content, soil with a bulk density of 1.48 Mg m^{-3}, weighted mean diameter of soil aggregates 0.74 mm, pH = 7.9, total C = 8.3 g kg^{-1}, total N = 0.83 g kg^{-1}, Olsen P = 28 mg kg^{-1}, and K = 128 mg kg^{-1}. Groundwater pumping is the predominant method of irrigation. Western UP has a diversified cropping system, with sugarcane-wheat and rice-wheat as the dominant cropping system. The crop was kept free of weeds by chemical spray. Observations on the desired parameters were recorded using the standard procedures. The main source of irrigation was canal water which was supplemented with tube well water as and when needed to meet the crop water requirements. The discharge available at outlet was measured every time. The time of irrigation application for each treatment was noted during each irrigation. The applied irrigation depth was calculated from measured discharge applied to known area for recorded time by the following equation:

$$QT = AD$$

Where Q = Discharge (Cusec,ft^3 s^{-1}); T = Time (h); A = Area (acres), and D = Depth (inches).

The amount of water (ft^3) applied to each treatment was determined by multiplying the discharge at field outlet with the time of application. The total amount of water so applied was computed for the entire crop season. The amount of water saved was determined by the difference of water applied to precisely leveled, unleveled and traditionally leveled experimental units. Water use efficiency was computed as follow:

$$WUE = Yield/Water\ applied\ (kgm^{-3})$$

Water productivity and economic analysis

Water productivity analysis combines physical accounting of water with yield or economic output to assess how much value is being obtained from the use of water (Molden et al., 2003; Abdullaev et al., 2007; Bouman et al., 2008). For this analysis, physical water productivity was calculated by:

$$WP = Output/Q$$

Where WP is the productivity of water in kgm^{-3}, output is the mass of crop in kilograms and Q is water resources applied and depleted (m^3). In this study, only physical productivities of the applied and depleted water are analyzed. To compare the laser-leveled field to the control field, both gross margin analysis and partial enterprise budgeting techniques were applied for three cropping seasons of 2009, 2010 and 2011.

The use of partial enterprise budgets required to evaluate technological innovations compared to old techniques, as the capital costs associated must be discounted over the life of the new investment.

RESULTS AND DISCUSSION

Yield and yield components

The laser leveling significantly affected the yield and yield components of rice, wheat and sugarcane crop (Tables 1 and 3). The maximum productive tillers were recorded in laser leveled field against the minimum in the unleveled field. No significant difference was recorded for 1000 grain weight. Laser land leveling produced maximum grain/cane yield (5.73, 4.60 and 82.8 t ha^{-1}) against the minimum (4.25, 3.85 and 54.9 t ha^{-1}) in unleveled field. Significantly higher grain/cane yield over traditionally leveled field and unleveled field might be attributable to better development of yield components like higher productive tillers m^{-1} row length and more 1000 grain weight due to more efficient use of inputs, uniform internode length, thicker canes and uniform availability of soil moisture in the effective root zone of the crop. Naresh et al. (2012) attributed higher grain yield in precision land leveling to more uniform “wattar” conditions that facilitated timely preparation of field and timely sowing of the crop as compared to unleveled fields. The reason for lower grain/cane yield in unleveled field might be uneven distribution of water over the field which drastically reduced the yield and yield components in lower and elevated spots.

Water saving

There was a significant improvement in irrigation performance when the precision laser land leveling was under taken prior to sowing (Tables 2 and 3). The maximum water depth for rice (122.4 mm), for wheat

Table 1. Rice - Wheat yield and its components as affected by laser land leveling and traditional leveling techniques.

Treatment	Plant height (cm)		Productive tillers (m^{-1} row length)		Grains $spike^{-1}$		1000 grain weight (g)		Grain yield (t ha^{-1})	
	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat
Laser land leveling	131.6	95.5	105	84	82	45	24.1	42	5.73	4.47
Traditional land leveling	127.7	87.4	97	68	77	43	23.4	39	5.35	4.23
Control (Unleveled)	111.8	76.1	84	59	68	39	21.3	39	4.25	3.75
CD at 5%	16.9	12.3	13.7	14.3	9.5	5.3	NS	NS	0.47	0.32

Table 2. Total duration, applied water depth and water use efficiency as affected by laser land leveling and traditional leveling techniques.

Treatment	Total duration min ha^{-1}		Water depth applied (mm)		Water depth/ irrigation (mm)		Volume of water applied (m^{-3})		WUE kg m^{-3}	
	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat	Rice	Wheat
Laser land leveling	3049	1263	810	340	90	63	4316	3310	1.33	1.35
Traditional land leveling	3414	1456	950	392	101	73	4982	3842	1.07	1.10
Control (Unleveled)	4134	1857	1260	501	122	93	6118	4915	0.69	0.76
CD at 5%	385	298	183	97	17	15	873	786	0.21	0.23

Table 3. Sugarcane yield and total duration, applied water depth and water use efficiencies as affected by laser land leveling and traditional leveling techniques.

Treatment	Total duration min ha^{-1}	Water depth applied (mm)	Water depth/ irrigation (mm)	Volume of water applied (m^{-3})	yield (t ha^{-1})	WUE (yield /mm water/ha)
Laser land leveling	6386	1630	160	7500	77.3	1.03
Traditional land leveling	7353	2046	185	8362	68.9	0.82
Control (Unleveled)	8382	2350	219	10363	55.7	0.53
CD at 5%	529	314	39	962	9.6	0.23

(100.3 mm) and (218.9 mm) for sugarcane were required to irrigate unleveled field during each irrigation as against the minimum in the field precisely leveled by laser and followed by traditionally leveled field. On an average, 36 to 12% in rice, 47 to 15% in wheat and 36 to 15% in sugarcane crop as compared to control and traditionally leveled fields reduced the total irrigation duration and water depth in each irrigation event, respectively. Thus, laser leveled field utilized less water per irrigation. The precisely leveled and smooth field showed a positive impact on the total water use resulting in a tangible reduction. At uniform discharge, before and after laser land leveling there was about 32% saving in water over control and 13% over traditional leveled field. Significantly, higher amount of water (6118.4; 4915.1 and 10362.8 m^3) were required for unleveled field than laser leveled field (4316.2, 3310.3 and 7500.4 m^3), which did not differ significantly from the traditionally leveled field. The results further revealed that 1802.2 m^3 in rice crop, 1604.8 m^3 in wheat crop and 2862.4 m^3 in sugarcane crop, that is, about 42, 47 and 38% excess volume of water was required to irrigate unleveled fields as against 15% (666.2; 531.7 and 861.9 m^3) in traditional leveled field. The only reason for excessive water application in control treatments was uneven surface to the unleveled treatment. The greater variation in surface level on unleveled and traditional leveled field resulted not only in wastage of water but also reduced crop yield by about 26 to19% in rice crop, 28 to 11% in wheat crop and 28 to 15% in sugarcane crop, respectively.

For rice:

Total duration	LL vs TL	3049.6 – 3414.7	= - 365.1 (12%)
	LL vs UL	3049.6 – 4134.3	= - 1084.7 (36%)
Water depth per irrigation	LL vs TL	90.3 – 101.5	= - 11.2 (12%)
	LL vs UL	90.3 – 122.4	= - 32.1 (36%)
Water use efficiency	LL vs TL	1.33 – 1.07	= 0.26 (21.8%)
	LL vs UL	1.33 – 0.69	= 0.64 (48.1%)

Table 4. Comparative energy and economics of laser land leveling and traditional leveling techniques.

Parameter	Laser land leveling		Traditional land leveling		Control (Unleveled)	
	Rice	Wheat	Rice	Wheat	Rice	Wheat
Energy requirement, MJ ha^{-1}	4768	2498	5658	2885	6960	3647
Tillage cost, Rs ha^{-1}	11675	8470	12450	10350	14370	12675
Grain yield, t ha^{-1}	5.73	4.47	5.17	4.23	4.25	3.75
Straw yield, t ha^{-1}	-	6.05	-	5.12	-	4.25
Gross income, Rs ha^{-1}	65,895	49,301	61,525	46,045	48,875	40,031
Cost of production, Rs ha^{-1}	24,175	18,720	25,700	19,578	27,425	21,460
Net income, Rs ha^{-1}	41,720	30,581	35,825	26,467	21,450	18,571
Benefit : cost ratio	2.73	2.63	2.08	2.35	1.78	1.87

For wheat:

Total duration	LL vs TL	1263.05 -1456.3	= - 192.95 (15%)
	LL vs UL	1334.05 – 1857.9	= - 594.85 (47%)
Water depth per irrigation	LL vs TL	63.2 – 72.9	= - 9.7 (15%)
	LL vs UL	63.2 – 93.1	= - 29.9 (47%)
Water use efficiency	LL vs TL	1.35 – 1.10	= 0.25 (18.52%)
	LL vs UL	1.35 – 0.76	= 0.59 (43.7%)

LL = Laser land leveling TL = Traditional land leveling and UL = Unleveled.

Water use efficiency

Water use efficiency (WUE) was significantly affected by different land leveling techniques (Tables 2 and 3). The highest WUE for rice, wheat and sugarcane crops (1.33, 1.35 and 1.03 kg m^{-3}) were recorded in laser-leveled field against the lowest (0.69 and 0.76 kgm^{-3}) in unleveled field while in traditionally leveled field were (1.07, 1.10 and 0.82 kg m^{-3}). Overall, the water-use efficiency was 48, 47 and 49% higher precisely in leveled field than control and 22, 19 and 20% higher than traditional leveling. This huge difference in water use efficiency was because of reduced grain/cane yield and higher amount of water applied to unlevel and traditional leveled fields. The decrease in water use efficiency in unleveled fields also reflected the sensitivity of the crop to water excess/deficit, a characteristic of undulating fields' surface of unleveled fields.

The reason for lower WUE in traditionally leveled and unleveled fields was the inefficient use of the water applied. The result suggests that laser land leveling is more water use efficient, more cost effective and give higher crop yield through efficient utilization of scarce land and water resources. Thus in the light of this study, it is imperative to recommend that laser land leveling should be popularized among the farmers at it not only increase water use efficiency and yield but also ensure better germination, better utilization of water and non water inputs towards increased yield.

For sugarcane:

Total duration	LL vs TL	6385.6 – 7352.7	= - 987.1 (15%)
	LL vs UL	6385.6 – 8682.1	= - 2296.5 (36%)
Water depth per irrigation	LL vs TL	160 – 184.5	= - 24.5 (15%)
	LL vs UL	160 – 218.9	= - 58.9 (36%)
Water use efficiency	LL vs TL	1.03 – 0.82	= 0.21 (20.4%)
	LL vs UL	1.03 – 0.53	= 0.50 (48.5%)

LL = Laser land leveling TL = Traditional land leveling and UL = Unleveled.

Profitability

Using scarce water resources in rice, wheat and sugarcane cultivation in a sustainable manner, brought a larger area under rice-wheat and sugarcane cultivation; the laser land leveling fields irrigation of these crops appeared to be an eco-friendly and economically viable technology. It led to higher productivity in rice, wheat and sugarcane and increased sucrose content in sugarcane and ultimately increased income for the farmers (Tables 4 and 5). Higher net returns were observed by laser land leveling technology Rs. 41,720; 30,581 and 66,280 ha^{-1} in rice, wheat and sugarcane crops in comparison to control (unleveled) fields. Other benefits include saving on fuel expenses, improvement in fertilizer use efficiency, uniform internode length, thicker canes, less weed growth and uniform irrigation of rice/wheat/sugarcane grown on undulated terrains.

Although, laser land leveling is beneficial, there are certain limitations associated with it such as high cost of the equipment/laser instrument and need for a skilled operator. It may be less efficient in irregular and small sized fields. Utilizing these eco-friendly and economically viable options will go a long way in sustaining rice, wheat and sugarcane productivity and economizing water under conditions of ever-depleting water resources.

Table 5. Economics of some agronomic measures on sugarcane production (plant cane).

Parameter	Demonstrated agronomic measure		
	Laser land leveling	Traditional land leveling	Control (Unleveled)
Energy requirement, MJ ha^{-1}	8339	9670	12100
Cane yield (t ha^{-1})	77.3	65.9	55.7
Cost of production (Rs ha^{-1})	49,640.0	52,830.0	56,790.0
Gross return (Rs ha^{-1})	115,920.0	103,350.0	83,550.0
Net return (Rs ha^{-1})	66,280.0	50,520.0	23,760
Benefit : cost ratio	2.34	1.87	1.47

Over the past decade, researchers in association with farmers and entrepreneurs have been trying to overcome the problems of depleting water resources, diminishing input use efficiency, declining farm profitability, and deteriorating soil health by developing, evaluating and refining conservation and precision agriculture-based resource-conserving technologies for the sugarcane-wheat and rice-wheat system in western Uttar Pradesh. Recently, laser-assisted precision land leveling has shown promise for better crop establishment, water savings and enhanced input use efficiency. This study have shown the effect of rice, wheat and sugarcane planting on laser leveled fields increased yields (av. of 3 yrs) by 6.6, 5.4 and 10.9% on traditionally leveled fields.

The saving in irrigation water with precision-conservation were 13.4, 13.8 and 10.3% compared to traditional leveling field and 29.5, 32.7, 27.6% to unleveled fields in rice, wheat and sugarcane crop, respectively. Therefore, this study confirms that Precision-Conservation Agriculture (PCA) based crop management solutions seem to be promising options to sustain the irrigated sugarcane-wheat and rice-wheat systems of western U. P. on a long-term basis.

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Full Length Research Paper

Bambara groundnut/maize intercropping: Effects of planting densities in Southern guinea savanna of Nigeria

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A field experiment was conducted in 2010 and 2011 at the Teaching and Research Farm, Federal University of Agriculture, Makurdi, Nigeria. The aim was to determine the suitability of some landraces of bambara groundnut for intercropping at varying planting densities with maize. The experiment was a 2×3×3 split-split plot laid in a randomized complete block design with three replications. Intercropping decreased number of pods and grain yields of bambara groundnut component. Number of pods and grain yields increased with increased planting density. Productivity indices indicated that bambara groundnut/maize intercropping was productive, and maize was the dominant component. The marginal rate of returns for the best combinations was 116.13%, suggesting profitability of the intercropping systems.

Key words: Bambara groundnuts, maize, intercropping, planting density.

INTRODUCTION

Bambara groundnut [*Vigna subterranean* (L.) Verdc] occupies a peculiar position in the diet of the people of West Africa and some authors (Ocran, 1998; Mkandawire, 2007) have placed it as the third most important grain legume after groundnut and cowpea in the region. The annual world production of bambara groundnut is about 330,000 tonnes. A high percentage (45 to 50) of this is produced in West Africa. Nigeria is leading producer of bambara groundnut in the world, producing about 33,000 to 49,000 tonnes annually (PROTA, 2006). The crop is essentially grown for human consumption. Quedraogo et al. (2012) described bambara groundnut seed as a complete diet, making it a good supplement to cereal-based diets. Apart from the leaves that can be used for livestock feed (Massawe et al., 2002), bambara groundnut is grown primarily for its seeds, which are used in many types of food. Mature, dry seeds are boiled and eaten as a pulse. Dried seeds, either whole or split, are also mixed with maize or plantains and then boiled. The seeds may be ground into flour, spiced and steamed as “moimoi” or fried as “akara” (Kadams and Sajo, 1998; PROTA, 2006). The hardy nature of the crop and its drought tolerance may endear it for inclusion in climate change adaptation strategies.

Maize (*Zea mays* L.) is the most important cereal crop in the world after wheat and rice (Onwueme and Sinha, 1991). It is a major item in the diet of many tropical countries whereas in the temperate regions, maize is the main grain used for animal feed. In Nigeria, maize is consumed in many forms: as maize flour made into a thick paste (‘tuwo’) and eaten with soup, ‘ogi’ (pap) and ‘agidi’, boiled or roasted as fresh corn and eaten with or

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Table 1. Physical and chemical properties of the surface soil (0 to 30 cm) at the experimental site in Makurdi in 2010 and 2011.

Parameter	Makurdi	
	2010	2011
Sand (%)	74	71
Silt (%)	18	18
Clay (%)	8	11
Textural class	Sandy loam	Sandy loam
pH (H_2O)	6.30	6.50
Organic carbon (g kg^{-1})	8.82	6.62
Total N (g kg^{-1})	0.70	1.00
Available P (cmol kg^{-1} soil)	17.50	11.75
Ca^{2+} (cmol kg^{-1} soil)	4.60	4.00
Mg^{2+} (cmol kg^{-1} soil)	2.40	1.30
K^{+} (cmol kg^{-1} soil)	0.14	0.12
Na^{+} (cmol kg^{-1} soil)	0.65	0.78
ECEC (cmol kg^{-1} soil)	7.79	6.20

without groundnut, palm kernel, fried, etc. Maize is industrially important chiefly for the production of alcohol, oil and starch (Onwueme and Sinha, 1991).

Despite its vast potentials, bambara groundnut remains one of Africa's crops often neglected by science. Nigeria is believed to be one of the centres of origin of this crop, and possesses extensive bambara groundnut genetic resources (Tanimu and Aliyu, 1995). In Southern Guinea Savanna region of Nigeria, some of the popular landraces include, "Karo", "Okirikiri", "Adikpo","Kparuru", "Ikpeyiole" and "Carol". In this region, these landraces of bambara groundnut and some others are grown by subsistence farmers in small patches of land. It is regarded as women's crop in most cultures and frequently intercropped or mixed with cowpea, maize and sorghum (Mkandawire and Sibuga, 2002). One major constraint in bambara groundnut production in Southern Guinea Savanna is inadequate information on the type and intensity of mixtures with other crop types in the cropping systems practiced by farmers, although Ngugi (1995) had indicated that bambara groundnut would do well if intercropped with maize if the maize planting density was low. Planting density of bambara groundnut is often low (<100,000 plants/ha) on farmers' fields (Egbe et al., 2009) resulting in low yields. Mkandawire and Sibuga (2002) had reported a spacing of 30 × 30 cm (approximately 111,000 plants/ha) in Tanzania and 60 × 30 cm (55,000 plants/ha) in West Africa.

Research information on the optimal planting density of bambara groundnut when being intercropped with maize in Southern Guinea Savanna is lacking. There is also dearth of scientifically documented information on yield advantages and the profitability of the bambara groundnut/maize intercropping systems in Southern Guinea Savanna of Nigeria. The study reported here sought to fill this knowledge gaps.

The objective of the study was to investigate the suitability of some landraces of bambara groundnut for intercropping at different planting densities with maize in Makurdi, Nigeria.

MATERIALS AND METHODS

Experimental site

A field experiment was conducted for two years (2010 and 2011) at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi (latitude 07°45' to 07°50' N, longtitude 08°45' to 08°50' E, elevation 98 m above sea level, masl) in Benue State, located in the Southern Guinea Savanna of Nigeria. The experimental site received a total rainfall of 1115.3 and 1211.4 mm in 2010 and 2011, respectively. The soil was classified as Dystric Ustropept (USDA). The experiment was sited in the same location in each year.

Soil sampling and analysis

Ten core samples of soil were collected from different parts of the experimental field from a depth of 0 to 30 cm and bulked into a composite sample and used for the determination of the physical and chemical properties of the soil (Table 1) before planting.

Experimental design, treatments and cultural practices

The plot was manually cleared with machetes and ridged with hand hoes before laying the experiment as a 2 × 3 × 3 split-split plot set out in a randomized complete block design with three replications. The main plot treatments were made up of two cropping systems [sole cropping (bambara groundnut), maize *var.* QPM] and intercropping (bambara groundnut + maize), while the sub-plot treatments were three bambara groundnut landraces ("Karo" "Okirikiri" and "Adikpo",). The sub-sub-plot treatments comprised of three planting densities of bambara groundnut (200,000 plants/ha, designated as P1 and set out as 1 × 0.05 m × 1 plant/stand;

100,000 plants/ha, designated as P2 and set out as 1 × 0.1 m × 1 plant/stand; 50,000 plants/ha, designated as P3 and set out as 1 × 0.2 m × I plant/stand). The bambara groundnut landraces were obtained from the local markets in Ankpa (“Karo”), Otukpo (“Okirikri”) and Makurdi (“Adikpo”). The maize variety used was *var*. QPM obtained from the Seed Technology Centre of University of Agriculture, Makurdi, Nigeria. The maize was planted in both sole and intercropping at 40,000 plants/ha, set out as 1 × 0.25 m × 1 plant/stand. Intercropping was formed by planting bambara groundnut at the top of the ridge, while maize occupied the side of the same ridge in a 1:1 row arrangement. The gross plot was made up of four ridges, spaced 1 m apart and 3 m long (12 m^2), while the net plot had two ridges, 2 m long (4 m^2). Planting was done on the 5^{th} day of August in both years of the experiment. Each treatment plot received an equivalent of 300 kg of NPK: 15:15:15 fertilizer as basal dressing by broadcasting. The maize component was top-dressed with Urea at 100 kg/ha by side placement 6 weeks after planting (WAP). The experiment was weeded manually using small hand hoes at 3 and 6 WAP.

Data collection and analysis

The following data were collected:

(1) Bambara groundnut component: Number of pods per plant and seed yield (t/ha),
(2) Maize component: Dry shoot weight and grain yield (t/ha).

The productivity indices used to estimate the intercrop advantage were:

(a) Land equivalent ratio (LER), an accurate assessment of the biological efficiency of the intercropping situation (Ofori and Stern, 1987), was estimated as:

$$LER = (X_{ab}/X_{aa}) + (X_{ba}/X_{bb})$$

Where X_{aa} and X_{bb} are yields as sole crops of bambara groundnuts and maize and X_{ab} and X_{ba} are yields as intercrops of bambara groundnuts and maize. LER figures greater than 1 are considered advantageous.
b) Land equivalent coefficient (LEC), a measure of interaction concerned with the strength of relationship (Adetiloye et al., 1983) was calculated thus,

$$LEC = PLERa \times P\ LERb$$

Where, PLERa = partial LER of bambara groundnuts and PLERb = partial LER of maize. For a two-crop mixture the minimum expected productivity coefficient (PC) is 25%, that is, a yield advantage is obtained if LEC value exceeds 0.25.
(c) Putnam et al. (1984) had reported that relative species competition was often evaluated using Competitive ratio (CR), which is a measure of the times by which one component crop is more competitive than the other. CR was calculated thus:

$$CRa = PLERa/PLERb \times zba/zab$$

Where CR*a* is the CR of bambara groundnut and PLER*a* and PLER*b* are the LERs of bambara groundnut and maize respectively, z*ba* is the proportion of bambara groundnut in the bambara groundnut/maize intercrop and z*ab* is the proportion of maize in the bambara groundnut/maize intercrop. If CR*a* < 1, there is a positive benefit and the crop can be grown in association; if CR*a* > 1, there a negative benefit. The reverse is true for CR*b.* d) Aggressivity as an index, represents a simple measure of how much the relative yield increase in crop ‘*a’* is greater than that of crop ‘*b’* in an intercropping system. It was computed as:

$$AGGab = (Xab/XaaZab) - (Xba/XbbZba)$$

Where, AGGab is aggressivity index, X*aa* and X*bb* are yields as sole crops of ‘*a’* and ‘*b’* and X*ab* and X*ba* are yields as intercrops of ‘*a’* and ‘*b’,* Z*ab* and Z*ba* are the sown proportions of ‘*a’* and ‘*b’*, respectively. If AGG*ab* = 0, both crops are equally competitive; if AGG*ab* is positive, ‘*a’* is dominant; if AGG*ab* is negative, ‘*a’* is the dominated crop (Ghosh et al., 2006).
(e) Profitability of the intercrop system was established by the results of net benefit analysis and marginal rate of return as described by CIMMYT (1988). These are indicators of monetary incomes accruing to the farmers from the intercrop system.

Year × treatment interactions were not significant, so data for both years were pooled together and analyzed. Data generated were analyzed using GENSTAT 13^{th} Edition. Means were separated by the use of the least significant difference (LSD) test at 5% probability level. T-test was also used where necessary for paired comparisons at 5% level of probability

RESULTS

The rainfall received within the experimental period was considered adequate for crop growth and development.

Bambara groundnut component

Number of pods per plant

The main effects of cropping systems and landraces were significant ($P \leq 0.05$), all other treatment effects were not ($P \geq 0.05$) on the number of pods per plant of bambara groundnut landraces intercropped with maize in Makurdi. Table 2 shows the main effects of cropping systems and landraces on the number of pods per plant of bambara groundnut intercropped with maize in Makurdi. The number of pods per plant of sole cropped bambara groundnut was significantly higher than the intercropped treatments. The number of pods per plant of “Okirikiri” (10.67) was significantly higher than “Karo” (5.94) and “Adikpo” (5.67).

Grain yield

All the treatments had significant effects ($P \leq 0.05$) on the dry grain yield of bambara groundnut intercropped with maize in Makurdi. Table 3 presents the effects of cropping systems × landraces × density on seed yield of bambara groundnut intercropped with maize in Makurdi. Grain yields of bambara groundnuts varied from 0.34 to 0.66 t/ha in sole systems and 0.23 to 0.53 t/ha under intercropping. Intercropping significantly lowered grain yields of bambara groundnuts. The grain yields of bambara groundnut landraces significantly decreased with declined planting density under both cropping systems. The grain yield of “Adikpo” in both sole (0.53 t/ha) and intercropping systems (0.43 t/ha) was significantly higher than “Karo” and “Okirikiri”.

Table 2. Main effects of cropping systems (CRS) and landraces on the number of pods per plant of bambara groundnut intercropped with maize in Makurdi.

Cropping systems	Number of pods/plant
Sole cropping	8.85
Intercropping	6.00
Mean	7.42
FLSD (0.05)	1.24
Landraces	
Karo	5.94
Okirikiri	10.67
Adikpo	5.67
Mean	7.42
FLSD (0.05)	3.00

Table 3. Effects of cropping systems (CRS) × landraces (VAR) × density (POP) on seed yield (t/ha) of bambara groundnut intercropped with maize in Makurdi.

Cropping systems	Landraces	P1	P2	P3	Mean
Sole cropping	Karo	0.54	0.44	0.34	0.44
	Okirikiri	0.55	0.43	0.36	0.44
	Adikpo	0.66	0.50	0.45	0.53
Mean		0.58	0.45	0.38	0.47
Intercropping	Karo	0.37	0.28	0.25	0.30
	Okirikiri	0.51	0.24	0.23	0.32
	Adikpo	0.53	0.42	0.35	0.43
Mean		0.47	0.31	0.27	0.35
CV (%)					
CRS	1.7				
CRS × VAR	5.1				
CRS × VAR × POP	5.2				
FLSD (0.05)					
CRS	0.02				
VAR	0.03				
POP	0.01				
CRS × VAR	0.03				
CRS × POP	0.02				
VAR × POP	0.03				
CRS × VAR × POP	0.04				

Maize component

Maize grain yield and dry shoot weight

Sole maize gave higher grain and dry shoot yields than intercropped treatments (Table 4). However, grain yield of sole maize and those intercropped with “Okirikiri” at P1 and “Adikpo” at P3 were at par. Also, dry shoot weight of sole maize was only significantly higher than those of intercropped maize with “Karo” at P3 and “Adikpo” at P1 and P2. The highest intercrop grain yield was obtained by “Okirikiri” landrace at P1 (2.21 t/ha), while the lowest was by “Okirikiri” at P2 (0.85 t/ha).

Land equivalent ratio, land equivalent coefficient, competitive ratio and aggressivity of intercropped Bambara with maize in Makurdi

Table 5 presents the LER, LEC, CR and aggressivity

Table 4. Dry shoot weight at harvest (t/ha) and grain weight (t/ha) of maize intercropped with landraces of bambara groundnut in Makurdi.

Treatment	Grain yield	Dry shoot weight
Sole maize	2.83	16.40
Maize in Karo at P1	1.46	12.58
Maize in Karo at P2	1.73	16.29
Maize in Karo at P3	1.42	14.40
Maize in Okirikiri at P1	2.21	16.21
Maize in Okirikiri at P2	0.85	15.65
Maize in Okirikiri at P3	1.69	16.06
Maize in Adikpo at P1	1.31	13.40
Maize in Adikpo at P2	1.53	13.34
Maize in Adikpo at P3	2.12	14.95
Mean	1.72	14.93
CV (%)	32.30	6.30
FLSD (0.05)	0.95	1.60

Table 5. Land equivalent ratio (LER), land equivalent coefficient (LEC), competitive ratio (CRa, CRb) and aggressivity (AGGa, AGGb) of bambara groundnut intercropped with maize in Makurdi.

Treatment	LER	LEC	CRa	CRb	AGGa	AGGb
Karo in maize at P1	1.20	0.35	0.26	18.70	-3.34	3.34
Karo in maize at P2	1.25	0.39	0.52	6.00	-1.35	1.35
Karo in maize at P3	1.26	0.38	0.38	2.07	-0.54	0.54
Okirikiri in maize at P1	1.69	0.71	0.23	21.30	-4.42	4.42
Okirikiri in maize at P2	1.22	0.37	0.14	6.99	-1.18	1.18
Okirikiri in maize at P3	1.25	0.39	0.28	1.42	-0.34	0.34
Adikpo in maize at P1	1.27	0.37	0.35	14.28	-3.95	3.95
Adikpo in maize at P2	1.38	0.45	0.78	3.86	-1.88	1.88
Adikpo in maize at P3	1.50	0.56	0.26	1.56	-0.34	0.34
Mean						
FLSD (0.05)	0.005	0.004	0.009	0.15	0.03	0.24
Paired t-test (0.05) CRa vs CRb				-3.17*		

a = Bambara groundnut; b = maize. ***,** significant at 5% level of probability.

values of bambara groundnuts intercropped with maize in Makurdi in 2010 and 2011. All intercrop treatments produced LER values above unity and LEC values beyond 0.25. Okirikiri combined with maize at P1 had the highest LER and LEC figures of 1.69 and 0.71, respectively. Karo at P1 produced the lowest LER figure (1.49). CRa was < 1 in all the intercrop combinations, but CRb was >1. While the CR of maize (CRb) decreased with decline in planting density of bambara groundnut, the CR of bambara groundnut (CRa) had erratic response. Aggressivity values of intercropped bambara groundnut landraces with maize were all negative, while the maize component of the intercropping had positive values.

Economic analysis

Total variable cost (TVC), gross returns (GM), net benefits, and dominance and marginal analyses

TVCs of intercropped treatments were significantly higher than sole crop (Table 6). Bambara groundnut landraces intercropped at P1 with maize had the highest TVC (N144, 421.00/ha), while intercropping at P3 gave the lowest TVC (N120, 796.00) (Table 6) with a mean of N131, 296.00/ha. Gross returns ranged from N223, 960.00/ha ("Okirikiri" at P3) to N330, 200.00/ha ("Okirikiri" at P1) (Figure 1) with a mean of N274, 097.78/ha. Gross returns decreased with decline in planting density of

Table 6. TVCs and net benefits of sole and intercrop treatments of bambara groundnut with maize.

Treatment	TVC (N/ha)		Net benefits (N/ha)	
	Sole crop	Intercrop	Sole crop	Intercrop
Karo at P1	87,500.00	144,421.00	52,900.00	134,179.00
Karo at P2	71,750.00	128,671.00	42,650.00	126,529.00
Karo at P3	63,875.00	120,796.00	24,525.00	119,004.00
Okirikiri at P1	87,500.00	144,421.00	55,500.00	185,779.00
Okirikiri at P2	71,750.00	128,671.00	40,050.00	126,769.00
Okirikiri at P3	63,875.00	120,796.00	29,725.00	103,164.00
Adikpo at P1	87,500.00	144,421.00	84,100.00	165,899.00
Adikpo at P2	71,750.00	128,671.00	58,250.00	167,489.00
Adikpo at P3	63,875.00	120,796.00	53,125.00	156,404.00
Mean	74,375.00	131,296.00	48,980.56	142,801.18
Maize	111,296	-	103,936.00	-
Paired t-test(0.05) Sole vs intercrop	4.17*		15.85*	

*, Significant at 5% level of probability.

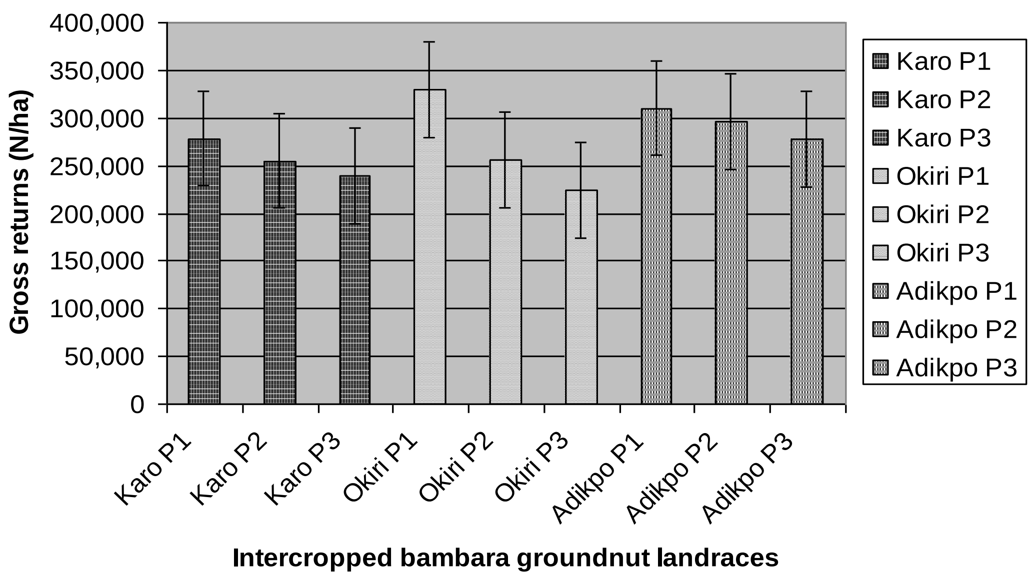

Figure 1. Gross returns of intercropped bambara groundnut landraces with maize at varying densities.

bambara groundnuts landraces (Figure 1). Net benefits of sole cropping were significantly lower than their intercrop counterparts (Table 6). Net benefits varied from N103, 164.00 (in "Okirikiri" at P3) to N185,779.00 ("Okirikiri" at P1). All intercrop combinations with maize were dominated, except "Adikpo" at P3 and P2 and "Okirikiri at P1 (Table 7). The marginal rate of returns for the best two treatments was 116.13% (Table 8).

DISCUSSION

The reduction in the number of pods per plant and the grain yield of intercropped bambara groundnut landraces as compared to sole cropping could be ascribed to inter-species competition for both under- and above-ground growth resources (water, nutrients, light, air, etc.). The taller maize component shaded the low canopy legume, thus reducing light availability for optimum photosynthetic activity and subsequently culminating in the low yields of bambara groundnut. Such observations are common in legume/cereal intercropping (Molatudi and Mariga, 2012; Alhassan et al., 2013). The yield of bambara groundnut in this study was generally low (0.23 to 0.66 t/ha) probably due to some inherent genetic properties/constraints, but Heuze′ and Tran (2013) had reported average yields of

Table 7. Dominance analysis of landraces of bambara groundnut intercropped at varying densities with maize in Makurdi, Nigeria.

Treatment	TVCs (N/ha)	Net benefits (N/ha)	Remark
Adikpo at P3 in maize	120,796.00	156,404.00	
Adikpo at P2 in maize	128,671.00	167,489.00	
Adikpo at P1 in maize	144,421.00	165,899.00	D
Okirikiri at P1 in maize	144,421.00	185,779.00	
Karo at P1 in maize	144,421.00	134,179.00	D
Okirikiri at P2 in maize	128,671.00	126,769.00	D
Karo at P2 in maize	128,671.00	126,529.00	D
Karo at P3 in maize	120,796.00	119,004.00	D
Okirikiri at P3 in maize	120,796.00	103,164.00	D

D, Dominated treatment.

Table 8. Marginal analysis of intercropped landraces of bambara groundnuts with maize at varying densities in Makurdi, Nigeria.

Treatment	TVCs	Total marginal costs	Net benefits	Marginal net benefits	Marginal rate of returns (%)
Adikpo at P3 in maize	120,796.00	7,875.00	156,404.00	11,085.00	140.76
Adikpo at P2 in maize	128,671.00	15,750.00	167,489.00	18,290.00	116.13
Okirikiri at P1 in maize	144,421.00		185,779.00		

300 to 800 kg/ha and also indicated that yields of up to 4 t/ha was possible. The low yields reported here further points to the fact that more work on genetic improvement of these popular landraces and some others in the region should be intensified to improve the productivity of the crop to enhance food security in the face of current climate change. The decline in grain yields with decrease in planting density in both cropping systems tested suggested that increased yields from increased planting density beyond 200,000 plants/ha was possible. It further signifies that the optimum planting density for these bambara groundnut landraces is yet to be reached, opening windows for further studies. Kouassi and Zorobi (2011) had reported that higher yields were often associated with higher planting densities (250,000 plants/ha) of bambara groundnuts in Cote d'Ivoire. Although yield depressions occurred, the maize component was more advantaged than the bambara groundnut. Trenbath (1976) had opined that the component of intercropping with its leaves held higher in the canopy structure was advantaged, particularly if the leaves were broad and horizontal. This was further proved by the positive aggressivity values of maize as compared to the negative values of the Bambara groundnut landraces. The positive aggressivity figures of maize showed that it was the dominant component of the intercropping, while bambara groundnut was dominated. Ghosh et al. (2006) stated that a positive aggressivity value meant dominance of a component crop in an intercrop arrangement. The CR figures of the bambara groundnut were significantly lower than those of maize, indicating superiority in competition by the maize component. Because adequate fertilizer was applied, maize plant height and biomass production were accelerated and subsequently its competitive abilities were enhanced. Egbe (2005) had observed similar results in pigeon pea/maize and pigeonpea/sorghum intercropping studies at Otobi and ascribed this superiority to height and biomass production advantage of the cereal component. The LER (>1) and LEC (>0.25) values obtained in this study proved that intercropping was advantageous. The intercrop advantage was due mainly to the greater grain yield of the maize component. Similar observations had been reported by Karikari (2003) in bambara groundnut intercropping with sorghum in Botswana. In this study, intercrop advantage was highest in Okirikiri at P1. This might be because maize combined with Okirikiri at P1 produced highest intercrop yield. Okirikiri combined with maize at P1 also produced the highest total gross returns and net benefits. Dominance analysis also showed that Okirikiri at P1 along with Adikpo at P2 and P3 were the only treatments not dominated. From the foregoing, Okirikiri at P1 proved to be one of the most suitable intercrop treatments in this study. Marginal analysis showed that intercropping Adikpo at P2 and P3 with maize and Okirikiri at P1 proved most profitable. Cropping systems strongly affected the net benefits derived from these intercrop combinations of bambara groundnuts and maize. The differences in yields, net benefits and marginal rate of

returns of these landraces of bambara groundnuts under intercropping with maize further emphasizes the importance of evaluating crop varieties before making recommendations crop varieties for intercropping.

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Review

Policy interventions and public expenditure reform for pro-poor agricultural development in Nigeria

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In Nigeria, agriculture and poverty are closely interlinked because poverty is higher in rural areas where agriculture is most practiced. As a result, changes in public policy and expenditure to agriculture can make a significant contribution to achieving poverty reduction and broader Millennium Development Goals. This requires a set of public actions and investments to foster broad-based growth in agriculture and other forms of rural enterprise. This paper reviews policy and expenditure reforms in Nigerian agriculture, what has driven these reforms, and the main areas of debate that are likely to be germane to transformation agenda of the present Government.

Key words: Policy intervention, pro-poor, public expenditure, Nigeria.

INTRODUCTION

Governments have traditionally intervened in agricultural sector in order to improve sector coordination and efficiency. Today, almost all countries in the world, including most high income countries, have forms of agricultural protection and subsidies with, reform proponents argue, often huge costs to the rest of the economy. Two reasons for this, especially in Nigeria, are the rising rural-urban income gaps, which can result in severe political tensions, and the demand for cheap agricultural products by urban populations. Agricultural sector reform aims at enhancing efficiency and reducing the cost to public agencies. Reforms involving withdrawal of marketing boards and other parastatal agencies aim at creating an environment which will foster the development of private competitive markets in agriculture and marketing. Reforms are also motivated by fiscal sustainability concerns in cases where parastatals are a financial drain on public agencies. Agricultural reforms remain an important part of national strategies, featuring strongly in most Poverty Reduction Strategy Papers (PRSPs) in Nigeria. A number of factors determine the public agenda in agriculture. These include the global context (international trade, regulatory standards), shifting ideological positions (state versus market-driven development), and national strategic priorities. At the national level, policy choices and the prioritisation and management of public funds to agriculture are determined by the following main elements:

i) An overall strategic framework for agriculture that sets out the role of agriculture in achieving national goals (growth, poverty reduction, environmental sustainability);
ii) A clear role for government in the sector relative to that of other players, most notably the private sector and NGOs, but also between Federal and other governments; and;
iii) The allocation and management of public funds to agriculture that is: (I) affordable and consistent with the resources available; (ii) in line with sector and national priorities, and (iii) ensures value for money.

Agriculture is also dependent upon non-agricultural policy
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and expenditure decisions that are beyond the control of ministries of agriculture - for example, spending on rural infrastructure (roads, irrigation), land reform policy,

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education and health services.

Over the last thirty years, the policy agenda in agriculture has been dominated by a shift away from state-led development - involving pervasive state intervention in agricultural production and marketing - towards the adoption of market-led approaches. This underpins the abolishment of Marketing Boards in Nigeria. This change has been driven by a number of factors; most notably, by an ideological shift towards economic efficiency and market-based solutions for resource allocation, combined with budget constraints and perceptions of greater benefits from spending to the social sectors. This perspective is supported by the poor performance of previous public spending in agriculture. A case in hand is the mis-appropriation of billions of Naira allocated to Kaduna Agro-Chemical Company for fertilizer production, which eventually led to total sales of the institution to private interest.

While these reforms has been undertaken by different administrations (Military and Civilian), the overall picture is not encouraging. Few reforms have delivered the results hoped for, in terms of either agricultural growth or rural poverty reduction. Meanwhile, public expenditure allocations to agriculture are static or declining. For instance, the percentage of budgetary allocation into agriculture between 1985 and 2005 was highest in 1999 at 3.40% of the total budgetary expenditure while the least of 0.5% was in 1985 and 2003. Nigeria has never met with the FAO and AU recommendation of 25 and 10% respectively. The declining trend in public spending to agriculture in Nigeria has occurred despite evidence that spending on certain public goods (rural infrastructure, research) contributes strongly to agricultural growth and poverty reduction. This raises serious questions for governments and donors about how future agriculture policy and expenditure reform should proceed. This study was therefore designed primarily to profile the performance and the effects of government agricultural reforms and strategies on the agricultural sector. Specifically the paper sought to: Review the various federal agricultural reforms, programmes and interventions designed and implemented to restore agriculture to its prime position in the economy; assess the effects and nature of the individual contributions of these reforms and programmes on the agricultural sector; profile and evaluate budgetary allocations to agriculture from 1960 to 2005, and compare it with African Union (AU) agreement; outline ways to making agricultural policies and programmes interventions pro-poor and conclude with recommendations for improvement.

Study area

Nigeria was the focus of the study. It has an area of 923,769 km^2 and a population of over 140 million people. It is bounded on the West by the Republic of Benin and the Republic of Niger; on the East by the Republic of Cameroon; on the North by Niger and Chad Republic's and on the South by the Gulf of Guinea. The climate is equatorial and semi-equatorial. There are two seasons; the wet and dry season and agriculture is a major employer of labour, and the mainstay of the economy despite her dependence on oil.

Secondary data of Nigeria budgets and budgetary allocation to agriculture obtained from the Central Bank Statistical Bulletin was used for the years 1960 - 2005. Budgetary allocation to agriculture was compared with the AU and FAO recommended per cent budgetary allocation. Descriptive statistics such as mean, variance, and percentage was used to analyse the data.

OVERVIEW OF AGRICULTURE POLICY AND EXPENDITURE REFORMS

In order to revamp the agricultural sector, the federal government had embarked on and implemented several agricultural policies and programmes some of which are defunct or abandoned, and some restructured while others are still in place. These include the farm settlement scheme, National Accelerated Food Production (NAFPP), Agricultural Development Projects (ADPs), River Basin Development Authorities (RBDAs), National Seed Service (NSS), National Centre for Agricultural Mechanisation (NCAM), Agricultural and Rural Management Training Institute (ARMTI) and Agricultural Credit Guarantee Scheme Fund (ACGSF). Others were the Nigerian Agricultural Cooperative and Rural Development Bank (NACRDB)/Agricultural Bank, Operation Feed the Nation (OFN), Green Revolution Programme, Directorate of Foods, Roads and Rural Infrastructure (DFFRI), Nigerian Agricultural Insurance Company (NAIC), National Agricultural Land Development Authority (NALDA), Specialised Universities for Agriculture, Root and Tuber Expansion Programme (RTEP) and rural banking scheme, etc.

Furthermore, the Federal Government in 2004 launched another economic reform called National Economic Empowerment and Development Strategy (NEEDS) programme to encourage private sector participation in the development of the economy. It was also aimed at promoting growth and poverty reduction through a participatory process involving civil society and development partners. In the agricultural sector, NEEDS were directed to influence improvement in the production, processing and distribution of agricultural commodities. NEEDS was short-lived for only one year and therefore could not transform or make significant impact on the agricultural sector.

Different strategies adopted by the country shows dynamism and changing strategies that overlaps and cannot be appropriately segregated into time phases. Often it was a combination of two or more strategies to implement agricultural policies designed at different time periods. According to Olayemi (1998) agricultural development strategies that have been adopted in the country can be categorised into the exploitative strategies, the agricultural project strategy, the direct government production strategy and the integrated rural development strategy.

Exploitative strategy

The Nigerian Government during the colonial period and early years of independence adopted this strategy for agricultural development. In the 1950s the traditional economists observed agricultural sector as a residual, subsistence sector made up of peasant farmers. Myint (1958) in his "Vent-for-surplus" theory particularly categorized a developing economy as consisting of a "modern sector" that is largely non-agricultural and a "subsistence sector" that is agricultural.

The subsistence sector perceived to be unproductive but full of under-utilized resources is expected to feed the modern sectors. As such, the subsistence sector was expected to be taxed to finance the modern sector. This essentially was the basis of the agricultural strategy in the 1950s and the 1960s in Nigeria with levies on export crops providing revenue for government to develop the modern sector (Adubi, 2004). The Government established institutions such as the agricultural marketing board system to boost revenue generation efforts through taxing of peasant farmers that produce export crops such as cocoa, groundnut, palm produce, cotton, etc.

Agricultural project strategy

The period coincided with the time of internal self-government up till 1968. Government intervention in agriculture was minimal. The small-scale farmers in Nigeria bore the brunt of agricultural development efforts (Egwu and Akubuilo, 2007). Agriculture was seen as a sector that has appropriate linkage with other sectors and should be developed in complementarity with other sectors thereby affecting the needed forward and backward linkages. Agriculture was regionalized with the establishment of extension fields and research institutes. Regional public funds were invested in agriculture and there were new schemes such as farm settlement schemes (established to create modern literate farmers and promote agricultural development). Tree crop plantations, smaller farmer credit schemes, and Agricultural Development corporation projects were established to encourage development of tree crops.

Direct government production strategy

According to Olayemi (1998), this was merely a deepening of the process of direct government intervention and investment in agriculture. This period started in 1970 and coincided with the oil boom in Nigeria. There was massive Federal Government intervention and investment in agriculture. The reasons were first, the need for the rehabilitation and resuscitation of agriculture after the civil war. This demanded immediate huge investments by government in agriculture given that there was low capacity in the private sector. Second, the ideological imperatives in the world then favoured direct involvement of government in directing investments in agricultural business and allied activities (Adubi, 2004). The period witnessed direct involvement of governments in directing investments in agricultural production activities and the establishment of schemes and research institutes such as National Accelerated Food Production Project (NAFPP), Nigerian Agricultural Co-operative Bank (NACB), etc.

Integrated rural development strategy

The government realized in the mid-1970s that the strategy of direct agricultural production was not yielding the desired results. So, there was gradual shift to an agricultural development approach which involved the adoption of an integrated rural development strategy (Olayemi, 1998). Under this strategy, rural development was seen from a holistic perspective with agricultural development problems being only part of a larger rural development concern. This prompted the government to embark on multipurpose rural development programmes and implementing institutions such as the Agricultural Development Projects (ADPs), the River Basin Development Authorities (RBDAS), the Directorate of Food, Roads and Rural Infrastructure (DFRRI), the National Agricultural Land Development Agency, (NALDA), the Operation Feed the Nation (OFN), the Green Revolution (GR), etc. This integrated rural development strategy was also adopted during the Structural Adjustment Programme (SAP) era but with significant changes in institutional design, intensity of activities and modes of operation.

Effects of agricultural reforms, policies and programmes on the agricultural sector

The assessment of the effects of the agricultural reforms and policies on the agricultural sector is with respect to the fundamental roles, of agriculture, namely:

i. Provision of adequate food for a growing population and raw materials for industries.
ii. Provision of an expanding market for non-agricultural products
iii. Generation of savings for investment in agriculture as well as other sectors and release of surplus or under-

Table 1. Proportion of the Nigeria's annual budget in agriculture: 1985 to 2005.

Year	Total budget (₦' m)	Allocation to agriculture (₦' m)	% allocation to agriculture	FAO percent recommendation	AU percent recommendation
1985	198,901.00	1,018.10	0.50	25.00	10.00
1986	33,245.40	925.40	2.70	25.00	10.00
1987	53,114.30	394.30	0.70	25.00	10.00
1988	71,753.90	650.00	0.90	25.00	10.00
1989	97,254.00	1,062.60	1.00	25.00	10.00
1990	129,164.00	1,966.60	1.50	25.00	10.00
1991	109,008.40	672.30	0.60	25.00	10.00
1992	156,107.10	924.50	0.50	25.00	10.00
1993	394,104.90	2,835.30	0.70	25.00	10.00
1994	371,900.00	3,719.10	1.00	25.00	10.00
1995	515,488.90	6,927.70	1.30	25.00	10.00
1996	594,260.50	5,574.00	0.90	25.00	10.00
1997	794,330.00	7,929.60	0.90	25.00	10.00
1998	1,176,289.30	1,184.40	1.00	25.00	10.00
1999	1,140,911.00	38,259.80	3.40	25.00	10.00
2000	1,190,597.20	10,596.40	0.50	25.00	10.00
2001	2,632,171.70	64,943.90	2.50	25.00	10.00
2002	3,770,106.50	44,803.80	1.20	25.00	10.00
2003	3,056,965.00	16,045.20	0.50	25.00	10.00
2004	1,971,752.50	59,773.40	3.00	25.00	10.00
2005	4,662,483.70	90,798.20	1.90	25.00	10.00

Source: CBN Statistical Bulletin (2007).

utilized resources to other sectors.
iv. Generation of foreign exchange.

These are discussed in line with the historical periods of the various policy reforms and programmes as follows:

The 1960 to 1969 era (period of minimum government intervention)

During this phase, government intervention in agriculture was minimal. The small-scale farmers in Nigeria bore the brunt of agricultural development efforts (Egwu and Akubuilo, 2007). According to Olayemi (1995) government effort took the form of settling policies and creating institutions for agricultural research, extension and export crop marketing and pricing. Agricultural development during this period was equated as the withdrawal of surplus rural labour and transferring them to the industrial sector. Government established farm settlements and government research institutes and agricultural development corporations. This period witnessed visible decline in export crop production and mild food shortage. There was a decentralized approach to agriculture with initiatives being left to the regions and the states while Federal Government played a supportive role.

Regional governments were executing ad-hoc policies, programmes and projects. The effects of these reforms/policies on agricultural performance include increase in food supply short falls, and rise in retail food prices (Sanyal and Babu, 2010). The agricultural share of the GDP declined from 66% in 1959 to 50% in 1970. The decrease in export earnings and the increase in retail feed prices led to greater importation of food, which adversely affected the balance of payments during the late 1960s (Kwanashie et al., 1998). During the same period, agriculture maintained an average of about 56% GDP in the 1960s with about 63% in the first half of the decade (Tables 1 and 2). However, the foreign exchange earnings declined from 71% in 1964 to 41% in 1969.

The negative environmental effects of these policy reforms at this period, however, were noted to include increased deforestation of rain forests for cash crop production as well as loss of biodiversity including wildlife and indigenous plants.

The 1970 to 1985 era (period of maximum government intervention)

This phase was characterized by a change of policy from minimal government intervention to maximum in the agricultural sector. The oil boom featured in the era which brought about enormous financial investments in agricultural projects and institutions. Many agricultural

Table 2. Growth rate of Nigeria's annual budgetary allocation to agriculture: 1985 to 2005.

Year	Allocation to agriculture (₦' m)	Growth rate (%)
1985	1,018.10	
1986	925.40	-9.11
1987	394.30	-57.39
1988	650.00	64.84
1989	1,062.60	63.47
1990	1,966.60	85.07
1991	672.30	-65.81
1992	924.50	37.51
1993	2,835.30	206.60
1994	3,719.10	31.18
1995	6,927.70	86.25
1996	5,574.00	-19.54
1997	7,929.60	42.26
1998	1,184.40	-85.06
1999	38,259.80	3130.31
2000	10,596.40	-72.30
2001	64,943.90	512.80
2002	44,803.80	-31.01
2003	16,045.20	64.18
2004	59,773.40	272.50
2005	90,798.20	51.90
Total	361,004.12	-
Mean	14,043.12	-
Variance	421,057,420.40	-

policies and programmes were enunciated.

The fiscal policy launched during the era ensured that budgetary allocations to agriculture were substantially increased to accommodate capital and recurrent expenditures. However, large budget deficits were recorded. The capital expenditure on agriculture declined from 6.2% of total capital expenditure by the Federal Government in 1973 to 4.0% in 1985. The expenditure of state government followed similar pattern for the period under review (Egwu and Akubilo, 2007). Under Tax policy, income tax reliefs on incomes from new agricultural enterprises were pursued. While a unified wage structure for all public sector workers was put in place.

The Monetary policy launched during the era ensured that Agricultural loans were given at concessionary interest rate of 6% per annum. In 1980s it was raised to 9% per annum.

Establishment of schemes, institutions etc: The Nigerian agricultural and co-operative bank (NACB) was established in 1973 to facilitate the granting of credit to Nigerian farmers. In 2000, NACB was merged with People's Bank and Better Life for Rural Women Programme and christened Nigerian Agricultural, Cooperative and Rural Development Bank (NACRDB).

Mandatory sectorial allocation to agriculture: Commercial and Merchant Banks were mandated to extend a minimum of 6% of their loan portfolio to agriculture which was later increased to 12%. Rural banking scheme was launched in 1977 while the agricultural credit guarantee scheme was established in 1977.

Trade policy on abolition of export duties on scheduled export crops in 1973 in order to promote agricultural export trade was established. Liberalization of imports in respect of food, agricultural machinery and equipment was ensured. A summary of the micro-economic policies in Agriculture during the era were as follows:

1. Agricultural commodity marketing and pricing policy: In 1977, six national commodity boards were established which include; commodity boards for cocoa, groundnuts, palm produce, cotton, rubber and food grains.
2. Land use policy was promulgated by the Federal Government in 1978 vesting the ownership of all lands on the government as to giving genuine farmers access to farmlands.
3. Agricultural extension and technology transfer policy aimed at improving the adoption of improved agricultural technology by farmers with the national accelerated food

production project (NAFPP) and agricultural development projects (ADPs) as implementing agencies.
4. Input supply and distribution policy was promulgated to ensure adequate and orderly supply of agricultural inputs notably fertilizers, agro-chemicals, seeds, machinery and equipment as follows:

a) In 1975, Government centralized fertilizer procurement and distribution with numerous agro-service centres nationwide.
b) In 1972, Government created national seeds service (NSS) to produce and multiply improved seeds such as rice, maize, cowpea, millet, sorghum, wheat and cassava.

5. Agricultural input subsidy policy on fertilizer, seed (50%) agro-chemicals (50%) and tractor hiring services (50%).
6. *Agricultural research policy:* The policy was aimed at coordination and harmonization of agricultural research and extension linkage. Agricultural research council was established in 1971. The 1973 Decree empowered the Federal Government to take over all state research institutions. The 1975 reconstitution by the Federal Government of the Nigerian Agricultural research Institute network led to the establishment of 14 institutes which were later increased to 19 and the creation in 1977 of the National Science and Technology Development Agency to coordinate all research activities in Nigeria.
7. *Agricultural co-operatives policy*: In 1979, a Department of Agricultural Co-operatives within the Federal Ministry of Agriculture, Water Resources and Rural Development was created to actualize this policy aimed at encouragement of farmers to form co-operatives and the use of same for the distribution of farm inputs and imported food commodities.
8. *Water resources and irrigation policy* brought about the establishment of eleven River Basin Development Authorities in 1977 charged with the responsibility of developing Nigeria's lands and water resources.
9. Agricultural mechanization policy: The policy was instrumental to the creation of the Ministry of Science and Technology and the establishment of some Universities of Science and Technology; the operation of tractor hiring units in all the states of Nigeria, reduced import duty on tractors and agricultural equipment and implements, generalized and liberalized subsidies on farm clearing and establishment of a centre for agricultural mechanization.

In terms of effects of these agricultural reforms and policies on the agricultural sector, the imbalance in the flow of financial resources that characterized this period reflected in Nigeria's foreign trade. During this period imports rose by 46.5% more than the planned targets, with food, capital equipment and raw materials being, the fastest growing categories of imports. Food imports as a share of total imports increased from 7.67% in 1970 to 10.26% in 1979 (Osemeobo, 1992). At the same time as imports were increasing, agricultural production was suffering due to the latent impact of the civil war (1967 to 1970) and the drought of 1972 to 1974 that led to a massive loss of crops and livestock. Despite government efforts in agricultural production, the performance of the agricultural sector was poor in terms of its growth, its export value, its contribution to GDP, and its share in Nigeria's total export earnings.

There was rapid decline in agricultural production with large food supply gaps (Sanyal and Babu, 2010) with attendant rapid increase in food imports from 7.7% in 1970 to 10.3% in 1979.

The 1985 to 1990 era (structural adjustment programme (SAP) and post SAP period)

This era saw the Federal Ministry of Agriculture, Water Resources and Rural Development in 1988 produce an agricultural policy for Nigeria decreed to be operational for at least the next fifteen years. According to Ikpi (1995) the document embodied the following, among other policies;

a) Agricultural sector policies and strategies on food crops, livestock and fish production, industrial raw material (crop and by-products) production, and forest products and wildlife.
b) Policies on support services such as agricultural extension, technology development and transfer, agricultural credit; agricultural insurance; agricultural mechanization; water resources development; rural infrastructure; agricultural statistics and data bank; agricultural investment and management advisory services, and agricultural manpower development and training. The document assigned role and responsibilities to the three tiers of government, federal, state and local in the country. It also incorporated a mechanism for periodic policy review to allow for policy stability and perspective planning. With the adoption of Structural Adjustment Programme (SAP) in 1986, government admitted the failure of past policies to significantly improve the economy and reverse the declining trend of production in the agricultural sector. The Structural Adjustment Programme relied most especially on the agricultural sector to achieve the objectives of its far-reaching reforms on diversification of exports and adjustment of the production and consumption structure of the economy (Adubi, 2004).

Despite the aforementioned policy measures, the agricultural sector did not register significant overall growth for several reasons. First, SAP had more of an impact on the distribution of farm incomes than on agricultural growth and productivity (Kwanashie et al., 1998). Second, on average, real producer prices of tradable goods did not change significantly after the policy reforms. The decline in output of the export crop subsector contributed to a reduction in foreign exchange earnings that could affect the foreign exchange requirement of the agricultural sector. As a result of this reduction and subsequent loss of export earnings from crops, the country's dependence on crude petroleum export earnings between 1988 and 1992 increased substantially (Colman and Okorie, 1993).

In this phase also, which marked the SAP period, there was lower agricultural and economic growth with high rates of unemployment. Export earnings declined to less than 5% (Table 3) as well as widening gap in food supply and demand. Food prices increased from 2.6% in 1970 to 1979 period to almost 20% during 1980 to 1989. The environmental implications of these policy reforms were quite significant. During this period, there was increased deforestation with adverse impact on biotic resources, loss of biodiversity, increased desertification in arid areas and flooding in lowland areas. There was also evidence of increased use of chemicals and abuse of fertilizer use which led to soil degradation in certain agro-ecological zones. With respect to the index of real agricultural sector GDP between 1985 and 1990, it was fluctuating over these years. It was negative (-0.13 and -1.56) in 1985 and 1986 and positive (36.45, 36.35, 5.30 and 18.43) in 1987 to 1990 (Ugwu and Kanu, 2012).

The new millennium agricultural policies (1999 to 2009)

At the inception of the new democratic administration in May 1999 and shortly before then, several institutional changes were made in order to realize the sector's objectives and in line with its belief that agricultural and rural development are sin quo non for improved economic recovery (Olomola, 1998). These include the relocation of the Department of Co-operatives of the Ministry of Labour and its merger with the Agricultural Co-operatives Division of the Ministry of Agriculture, the transfer of the Department of Rural Development from the Ministry of Water Resources to the Ministry of Agriculture (all before 1999), the scrapping of the erstwhile National Agricultural Land Development Authority (NALDA) and the merging of its functions with the Rural Development Department, the scrapping of the Federal Agricultural Co-Ordinating Unit (FACU) and the Agricultural Projects Monitoring and Evaluation Unit (APMEU) and the setting up of Projects Co-Ordinating Unit (PCU) and later transformed into the National Food Reserve Agency (NFRA). Streamlining of institutions for agricultural credit delivery with the emergence of the Nigerian Agricultural Co-operative and Rural Development Bank (NACRDB) from the merger of the erstwhile Nigerian Agricultural and Co-Operative Bank (NACB) and the Peoples Bank and the Family Economic Advancement Programme (FEAP). New Institutions were also evolving to enable the Nigerian agricultural sector respond to the imperatives of the emerging global economic order.

The new agricultural policy has a clear statement of objectives. The policy seeks to attain self-sustaining growth in all the subsectors of agriculture and the structural transformation necessary for the overall socio-economic development of the country as well as the improvement in the quality of life of Nigerians. This objective reflects the current policy recognition of agriculture as a vital sector under the poverty reduction programme (FMARD, 2003).

Other initiatives that are on-going to step-up agricultural development and ensure food security are the Root and Tuber Expansion Programme, National Cocoa Development Committee, Special Programme on Food Security (SPFS) and National Fadama Project. Others also include the Fish Farm Estate Development, Initiatives for Increased Agricultural Production, South-South Cooperation Initiative and Nigeria-France Project on Agricultural Development.

This phase witnessed a drastic reduction in food imports from 14.5 to 5% of total imports. Presidential initiatives on specific agricultural commodities (for example cassava, rice) in order to generate N3 billion annually from exports also featured during this period. Public private partnership in the development of agricultural marketing as well as the promotion of integrated rural development marked this phase. The effects of these policy changes and programmes were reflected in the deficits recorded due to rising population and import restrictions on cereals and grains, supply shortages due to significant increase in land area under cultivation, and instability in input and output markets, among others. Annual deforestation rate remained at the rate of 76% per year due to higher demand for agricultural land, fuel wood and rapidly growing population (UNEP, 2006). Land degradation caused by soil erosion occurred at an alarming rate. However, Nigerian agriculture has shown good growth rates in the recent past with growth rates of 7.4, 7.2 and 6.5% in 2006, 2007 and 2008, respectively. Between 2003 and 2007 its average share of the national real GDP was 41.5% thus underscoring its importance in the livelihood of Nigerians (FGN, undated). Of the growth in the 2003 to 2007 period, the crop, livestock, fishery and fishery subsectors contributed 90, 6, 3 and 1% respectively. Major crops grown in Nigeria include yam, cassava, sorghum, millet, rice, maize, beans, dried cowpea, groundnut, cocoyam and sweet potato. These major crops which accounted for about 75% of total crop sales in 2004 increased from 81,276 thousand tonnes in 2004 to 95,556 thousand tonnes in 2007.

Problems/challenges of the agricultural reforms, policies and programmes

A critical examination of the reforms/policies and their implementation over the years show that policy instability, policy inconsistency, lack of policy transparency, poor coordination of policies as well as poor implementation and mismanagement of policy instruments constitute major obstacles to the implementation and achievement of the goals and objectives of these policies. Policy instability and lack of policy transparency are not unconnected with political instability and bad governance. For example, between 1979 and 1999 the country had five military/civilian regimes.

Many of the strategies used to improve agricultural growth in the past have failed because the programmes and policies were not sufficiently based on in-depth studies and realistic pilot surveys (Adebayo, 2004). This could be attributed to lack of public participation in the design, formulation, implementation and evaluation of policies as well as limited implementation capacity within the sectoral ministries and a poor understanding of the details and specifics of polices by implementers (Adebayo, 2004).

The main factors that influenced the effectiveness of policies on agriculture include high demand for agricultural produce, availability of improved technology, efficient dissemination of information by the ADPs and value added leading to improved income. On the other hand, the common factors responsible for the ineffectiveness of policies and regulations, especially on the downstream segment of agriculture, include instability of the political climate, insecurity of investment, non-standardized product quality, non-competitive nature of agricultural products from the country in the export market due to high cost of production and lack of adequate processing facilities (The New Nigerian Agriculture Policy, 2001).

Public expenditure trends

There have been many studies of the relationship between government expenditure and economic growth. Some of these have looked specifically at the link between government spending and agricultural growth and poverty reduction (Fan et al., 2000a, b). These studies show positive growth and poverty reduction effects from public spending in agriculture. Yet, in the majority of developing countries aid and public expenditure to agriculture is stagnant or declining. This is especially true of Nigeria.

Table 1 shows the percentage of budgetary allocation into agriculture between 1985 and 2005 in Nigeria. The highest percentage allocation of 3.40% was in 1999 while the least of 0.5% was in 1985 and 2003. On the overall, the years 1999 and above had higher percentages of budgetary allocation to agriculture than the years before. This implies that Nigeria did not meet the FAO and AU recommendation of 25 and 10% respectively. Perhaps this is partly responsible for the low performance of agriculture and high poverty level in the rural areas since the country's agriculture is rural based.

The mean of the budget distribution between 1985 and 2005 is N14,043,120,000.00 and the standard deviation is N20,519,683,730.00 as shown in Table 2. The budgetary annual growth rate was negative in 1986, 1987, 1991, 2000 and 2002 of the values -9, -57, -66, -20, -85, -72 and -31% respectively while it was positive for other years.

From the graph (Figure 1) above, the line graph on top represent the total budget of Nigeria between 1985 and 2005 while the near-flat line graph represent the budgetary allocation to Agricultural sector within the same time period. It is obvious from the graph that although total budget shows a positive trend as the slope seems to be positive until 2002 when a sharp drop in total allocation was observed, the slope of agricultural budget remains unnecessarily constant over the same period. Similarly, using a pie chart to illustrate this colossal allocation to Agricultural sector over the years will make a vivid statement on inadequate allocation to the sector.

From the above pie charts (Figure 2) representing total budget and Agricultural budgetary allocations over the years, it is definitely obvious that Agricultural budget is noticeable in 1988, 1999, 2001, and 2004. The alarming positive growth rate was in 1999 with 3,130.31% from 1998 to 1999. Per cent growth rate was 64 and 272 in 2004 and 2005 respectively. This shows improved commitment but below the international expectation judging from per cent allocation to the agricultural sector as shown in Table 1.

From Table 2, percent budget allocation to agriculture in 1985, 1992, 1996 and 2004 was 0.5, 0.5, 0.9 and 3.0 respectively. This is in contrast to the Maputo Declaration (2003) where the Heads of States of the Assembly of the African Union committed (2003) their support to the Comprehensive Africa Agriculture Development Programme (CAADP) and pledged to raise spending on agriculture to 10% of the budget within the next five years, by 2008. Also, at the second ordinary assembly of the African Union in Maputo in July 2003, African Heads of States and Governments endorsed the 'Maputo Declaration on Agriculture and Food Security in Africa'. This declaration came upon the recognition of the pivotal role of agriculture in the quest for good life and wealth creation, as one good way of addressing poverty and its attendant consequences is through agriculture (FMARD, 2010). The declaration contained several important decisions regarding agriculture, which included the commitment to the allocation of at least 10% of national budgetary resources to agriculture and rural development policy implementation within five years. Though this fell short of the 25% recommendation of Food and Agricultural Organisation (FMARD, 2010), several lessons can be drawn from the fore-going analysis:

i) Governments should consider increasing spending in

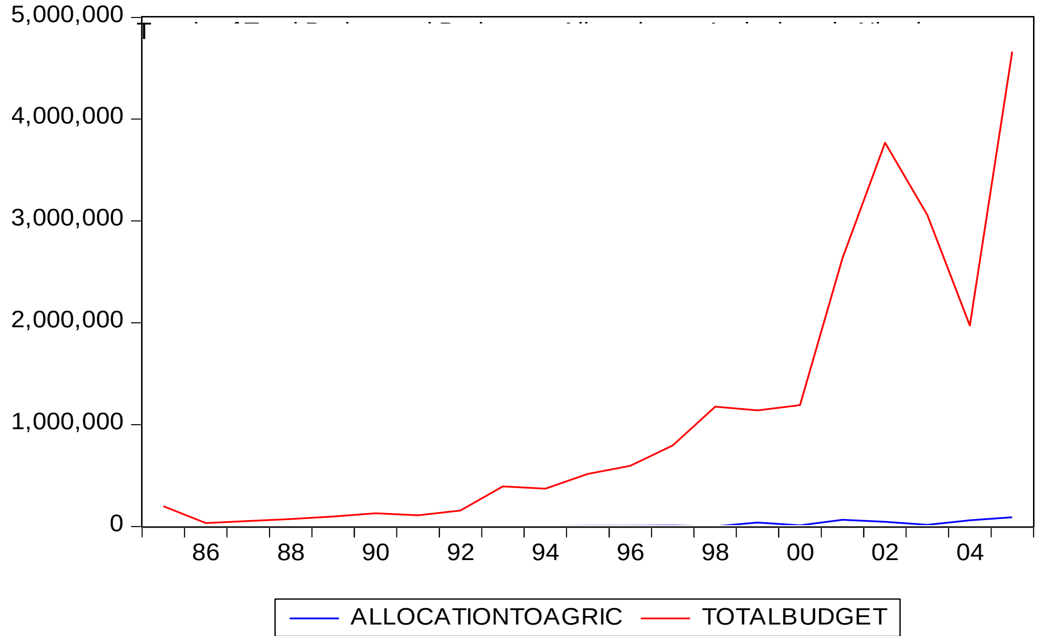

Figure 1. Trends of total budget and budgetary allocation to Agriculture in Nigeria.

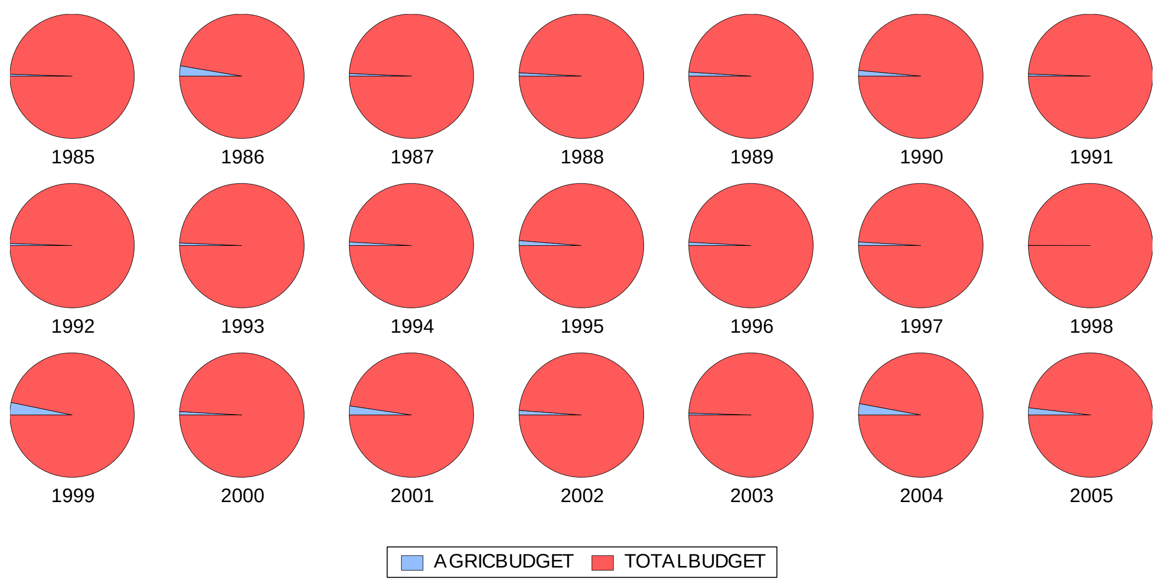

Figure 2. Total budget and Agricultural budgetary allocations over the years 1985 to 2005.

agriculture, particularly on production-enhancing investments such as agricultural research and rural infrastructure. This type of spending not only yields high returns to agricultural production, but also has a large impact on poverty reduction since most of the poor still reside in rural areas and their main source of livelihood is agriculture.

ii) For agricultural research, increased funding alone is not the answer - developing effective public-private partnerships and making more efficient and effective use of research funds present major challenges.

According to Dorward et al., (2002), despite potential positive returns to public spending in agriculture, few policy makers currently consider investment in agricultural development the best bet for poverty reduction. There are a number of reasons for this.

One, recognition that achieving agricultural growth in remote and marginal rural areas - where much of the rural poor are now concentrated – is more difficult. Two, the perception that many of agriculture’s problems are seen as lying outside the agricultural sector – for example, in roads and telecommunications infrastructure, in health and education. Three, uncertainty regarding how best to invest in agriculture. Much of the investments called for tend to focus on research and extension, but policy makers have doubts about their effectiveness, are concerned about recurrent costs and fiscal commitments, and are experimenting with private/public models for finance and delivery. Four, increasing recognition of the importance of non-farm incomes and activities to the livelihoods of the rural poor. This is a reason politicians would rather invest in Motorbike popularly known as “okada” rather than investing in agriculture in rural areas.

HOW TO ACHIEVE PRO-POOR AGRICULTURAL GROWTH

Much of the poor live in rural areas and many of these poor are farmers. This suggests that growth in agriculture is the best way to end poverty. But how can this growth be achieved?

In the 1960s, the secret to agricultural development was identified as investment in new agricultural technologies and human capital, and getting prices right. Correct price incentives in agricultural markets would generate profitable investments and income streams that would then increase commodity output and lift the rural economy out of poverty. However, the diversity of rural circumstances and the changing global environment have sharply impeded agricultural development and progress in reducing rural poverty. For example, neither the agricultural technology nor the incentive prices in agricultural markets have been reliably available. Success in linking the non-tradable sector in rural areas to urban markets and labour-intensive export growth has been mixed, with poverty becoming concentrated in remote rural areas where the benefits of wider economic growth are not felt.

The success of Asia’s ‘green revolution’ in the 1960’s has proven difficult to replicate in Nigeria. Green revolution in Nigeria was based upon productivity enhancing seed and fertiliser technologies, combined with high level of state spending in agriculture and high demand for cereals. Ensuring pro-poor agricultural growth is now more difficult. Nigeria has failed to keep pace with the new technologies, government cannot afford high levels of public expenditure in agriculture, and prices for staple cereals are escalating.

What does this mean for future reforms to agriculture policy and expenditure in Nigeria? The mechanisms for both technology development and provision of rural price incentives are no longer as clear as they were in the 1960s. In many circumstances the poor do not have access to productivity enhancing technologies. Where they do have access, higher agricultural productivity can lead to lower food prices – to the benefit of poor consumers who spend a large share of their budget on food, but unless increased productivity is matched with higher demand or employment opportunities in the non-farm sector, farmers can end up worse off. So using agricultural technology to solve problems of rural poverty is complicated at best.

Good governance provides an approach for addressing problems of weak agricultural growth. However, the speed and impact of improvements in governance has been less in rural areas due to lower levels of education, lower qualification of civil servants, and more deeply ingrained traditions of paternalism. The effectiveness of public sector institutions in promoting pro-poor agricultural growth is also hampered by the fact that there are often many different ministries or agencies operating within the sector (for example, public works, water resources, trade, and environment) each with a high degree of centralisation (Timmer, 2003).

Role of government in agriculture

The debate continues on the appropriate role for the state in agriculture. Many argue that market liberalisation and an increasing focus by the state upon correcting for instances of market failure have had little positive impact. Confusion also remains over how to define public goods in agriculture and appropriate levels and forms of state intervention to the sector.

The reasons for the limited impact of policy reforms and prescriptions to address it are debated. One view is that failure is not the result of policy reforms *per se*, but of failure to implement the reforms thoroughly (Jayne et al., 2001). Partial reforms that allowed parastatals to continue operating, together with policy reversals, have inhibited incentives for private sector investment. It is suggested that the solution is to complete the policy reform process, accompanied by appropriate supporting measures and public investments – for example, in public goods (rural infrastructure, market institutions), agricultural support organisations (research and extension), new approaches for service delivery (contracting out, group approaches), short term targeted support to vulnerable groups in remote areas (safety net transfers); and credible and sustainable macro-economic policies.

An alternative view is that high transactions costs in poor rural areas prevent the private sector from delivering necessary agricultural services. Transactions costs are especially high for financial services (as there is much associated risk), but also hinder the development of input and output markets. This analysis has two important implications. First, a policy of government withdrawal from service provision and the promotion of competitive markets may actually worsen the problems faced by smallholder farmers. Second, rural infrastructure and institutional development are insufficient on their own to attract the level of private sector investment necessary for growth in agriculture. Supporters of this view argue that governments should continue to pursue interventionist policies in order to remedy these market failures - until economic activity and institutional development proceed past a critical point (Dorward et al., 2002; Diao et al., 2003).

This suggests that arguments for state intervention in agriculture are particularly compelling where there are problems associated with weakly integrated markets, where conditions prevent markets from functioning effectively, or where the situation of the poor is such that they simply are unable to participate fully in markets. This is particularly the case in remote rural areas. Similarly, lack of competition between service providers may necessitate state intervention. The absence of competition between suppliers reduces the incentive for the provider to reduce costs and this may offset the advantages of private sector delivery. Here, public intervention in service provision or through regulation (to set conditions of competition, and pricing and quality standards) will be necessary.

For some, the concept of coordination failure suggests a possible role for government action that goes beyond the standard 'market failure' model of the public role in agriculture. A coordination failure can be defined generally as a case where "*individuals' failure to coordinate complementary changes in their actions leads to a state of affairs for everyone that is worse than some alternative state of affairs that is also an equilibrium*" (Hoff, 2000). In a more restricted but relevant sense for the issues under consideration here, an alternative definition refers to the "*failure of one's own investment due to an absence of complementary investments by other players at different stages in the supply chain*" (Dorward et al., 2003).

In the absence of mechanisms to overcome coordination failure and to supply assurances that complementary investments will occur, private initiatives will be sharply curtailed. Risks of strategic default on debt, or uncertainty about demand, can create a situation in which potentially viable sets of investment do not occur. This situation is pervasive in many rural situations where transactions costs are high and the institutional framework is weak, particularly for investments that are illiquid, long-term, and have few alternative uses (such as processing facilities for particular crops such as cassava or sugar) (Jones and Joffe, 2003). Others take a wider view, suggesting that market failure arguments to justify public interventions in agriculture are used too loosely and uncritically. While the theoretical literature defining market failures is generally not contentious, there is no clear methodology for identifying and quantifying market failures in practice, and for linking this identification to specific policy proposals. Hence, in practice, the market failure approach does not provide sufficiently rigorous criteria for determining the choice of policies or instruments (Van der Meer and Noordam, 2003).

Another perspective on market failure is the extent to which public intervention has exacerbated rather than improved the problem. Even in the post-adjustment era, a continued heavy-handed state presence is still evident in many rural economies, creating an environment in which layers of subsidies may distort and weaken incentives for private investment. Pervasive problems of political and economic instability, lack of transparency, corruption, irrational regulation and bureaucratic load also raise transaction costs and risks to business in Nigeria.

What types of public investment achieve agricultural growth?

Studies show that rural infrastructure and agricultural research provide the greatest returns to public investment in agriculture (but may not be sufficient alone to guarantee pro-poor agricultural growth). Although it is hard to argue that agricultural research has a direct impact on the poor in rural areas, as new technologies are often adopted earliest and most intensively by better-off farmers, the indirect effects of higher agricultural productivity are likely to have a positive impact on the poor.

There is also scepticism that returns to research for more marginal areas can match those of more favoured areas. Others suggest however, that marginal areas may offer greater returns to research investments simply by virtue of being neglected in the past (Lipton, 1988). There may also be significant returns to research on indigenous products in marginal areas the potential of which remains largely unknown and untapped.

Similar arguments can be made for public provision of rural infrastructure. Rural roads assist larger farmers to a greater extent than smaller subsistence-oriented farmers, but the productivity effects spread quickly and rapidly to the poor. The poor may also benefit through labour-intensive public works programmes in the construction of rural infrastructure.

As already noted, Fan and Rao (2003) show that government spending on agriculture has provided a strong contribution to economic growth in Africa and Asia, and that agricultural production is critical for addressing poverty in rural areas. This view is supported by Diao et al. (2003) who show that spending on rural infrastructure (especially roads) and productivity enhancing investments in agricultural export crops and livestock have the most promise for growth in income and food consumption in Africa. They also support the view that there are high returns to initiatives and investments that reduce transaction costs in agricultural markets. Reduced marketing costs are crucial to reducing consumer food prices, while also raising producer incomes in the longer term. Investments in infrastructure and policy reforms that lead to reductions in transaction costs have the potential to benefit a wide spectrum of agricultural (and non-agricultural) activities, and avoid the problem of requiring the government to 'pick winners' among competing crops or agricultural activities. However, without growth in the non-agricultural sector, overall gains will be limited. Investments in agriculture need to be complemented with policies and investments to spur non-agricultural growth.

Timmer (2003) suggests that once agricultural technology and efficient rural infrastructure are in place as the basis for profitable farming, policy attention and budget priorities should turn to the rural non-tradables sector. Part of the profitability for this sector will come from a labour-intensive export sector that is successfully linked into the global economy. Rapid growth in this export sector creates demand for labour directly as well as for the goods and services of the rural economy that raise demand for labour indirectly.

There is also an important role for government in providing a supportive policy and institutional framework that creates incentives for private sector investment in agriculture. Areas of action include institutional arrangements that support sustainable investments in infrastructure, land titling, regulatory capacity for rural financial systems, and the strengthening of farmer organisations and the capacity of rural local government. Some of these solutions lie within the rural sector – others depend on more general measures to improve the enabling environment for the private sector and the effectiveness of government expenditure. Where public action is used as a transitional measure to catalyse private sector investment and market development, it needs to create sustainable institutional development so that privately financed solutions may become viable. An example is where the intervention enables a 'coordination failure' to be overcome (Jones and Joffe, 2003).

Agriculture in PRSPS

The production by countries of a Poverty Reduction Strategy Paper (PRPS) is a key requirement for low income (IDA only) countries. PRSPs provide access to HIPC debt relief and are an important factor in determining levels of bilateral donor support.

Two issues can be highlighted. First, almost all PRSPs propose large increases in social sector spending - in particular, health and education. Norton and Foster (2001) question whether there is an overemphasis on these sectors at the expense of productive sectors, such as infrastructure and agriculture. While emphasis on health and education is relevant to poverty reduction, investment in the productive sectors is also essential, and may have a more substantial and direct impact.

Second, while all PRSPs include a chapter on Agriculture and the principal constraints facing the sector, few provide a convincing case for government actions to address these problems. A critical strategic choice that needs to be addressed is whether to concentrate agricultural development activities on high potential areas, where there are greater opportunities for increasing growth and national food supply, or low potential areas, where poverty and food security may be more severe. Most of the PRSPs do not clearly explain government policy in respect of this important trade-off. This is a neglected issue, which requires much greater analysis and strategic discussion (Williams and Duncan, 2001). Fortunately, Nigeria has evolved a dual strategy of increasing productivity in high potential areas, while attempting to tackle structural food insecurity in marginal and low potential areas. Such balanced approaches are likely to offer the best prospects for tackling food insecurity.

Public expenditure management

While agricultural policy reforms often imply a changed role for government, they do not necessarily reduce the case for public spending on agriculture. However, in order to make a convincing claim for public funds, Ministries of Agriculture need to demonstrate effectiveness and efficiency in what they do. In addition to the policy framework, this requires the strengthening of public expenditure management systems, especially the recording of data on agricultural expenditures and their impact. Assessing the composition, relevance, efficiency and impact of spending to agriculture (and to other sectors) is made difficult by weak public expenditure management and accountability systems. Systems of public expenditure management should be strengthened to ensure that: (i) the composition of public spending to agriculture is consistent with policy priorities; and (ii) Ministry of Agriculture are able to make a convincing claim for scarce public resources.

Direct budget support

In recent years there has been an increasing trend by donors towards direct budget support. This refers to the channelling of donor funds to a recipient government using the government's own allocation, procurement and accounting systems. While some budget support is tied to specific sector, much is in the form of general budget support without any formal limitation on sector allocations. The presumption is that it is government's responsibility to sector policy and budget priorities.

There is a concern that the shift towards direct budget support results in a diversion of public funds that otherwise would have been allocated to agriculture. It also distances donors from their responsibilities in implementing effective strategies to reduce poverty.

The recent DFID e-Forum on agriculture noted that one, direct budget support often results in a shift in decision-making processes and resource allocations that work against ministries of agriculture - often one of the weakest sector Ministries and the least capable of making a convincing case to Central Finance Ministries for scarce budget resources. Two, without targeting resources to agriculture it is difficult to ensure that the sector receives the resources needed to stimulate growth. It also makes it difficult for donors to provide effective technical support to Ministries of Agriculture to target poverty reduction. Technical assistance previously 'bundled' with agriculture sector investment projects is hard to target in isolation.

Reform of Ministry of Agriculture

Ultimately, strengthening the public role in agriculture and securing increased public funding to the sector, requires a strengthening of the bargaining power of Ministries (Federal and State) of Agriculture and other agencies involved in the sector. The weakness of Ministries of Agriculture, especially in sub-Saharan Africa, is seen by many as an obstacle to Agriculture fulfilling its potential as a driver for growth and poverty reduction (DFID e-Forum on Agriculture) DFID (2004).

It is also questionable whether hierarchic Ministries of Agriculture, mostly starved for funds, can be transformed into delivering services demanded by poor rural communities? This question is especially relevant for failing states. Some suggest that reform of Ministries of Agriculture is needed. Others suggest that perhaps a better approach would be to channel support through community organisations and NGOs. This raises the issue of effectively engaging the poor in policy processes. The mechanisms to allow effective participation by the poor in all stages of the policy process, whilst well known, are rarely used effectively.

CONCLUSION AND RECOMMENDATIONS

Two principal issues emerge from this paper. The first is that broad-based growth in agriculture is the key to achieving the Millennium Development Goals. However, achieving growth in agriculture is harder than it was previously. Taking forward the agenda requires a policy framework that goes beyond the traditional market failure approach, that recognises the important linkages between agriculture and the non-farm economy, and which allows a more dynamic understanding of the interaction between public and private roles.

Second, there has been a loss of confidence in public spending to agriculture. In response, there is a need to sharpen the administration and accountability of public funding to the sector. To make a convincing claim for public funds Agriculture agencies increasingly need to demonstrate effectiveness and efficiency in what they do. Based on the unresolved issues already highlighted, this paper suggests a number of ways germane in addressing both issues thus:

i) There is a need to revisit the role of government in agriculture. This could be informed by a review of agricultural policy reform experience with a focus upon identifying what has worked and why. The review should look at experience in establishing public and private roles in service provision and incorporate ideas about coordination and measures to reduce transactions costs in unfavourable institutional environments.

ii) Open and inclusive policy debate about the role of agriculture (especially smallholder agriculture) in economic growth and poverty reduction should be encouraged. The debate should be evidence-based and focussed around an understanding of the political economy for change, and public and private roles in making markets work.

iii) Decentralisation presents a significant challenge for agricultural service provision. It would be useful to review experience of decentralisation implementation in relation to its impact upon the quality of agricultural services provided and the extent to which it has promoted greater participation (especially by the poor) in policy processes and the prioritisation of public resources to agriculture.

iv) In considering how further reforms should be implemented, good governance and political economy considerations ('drivers of change') are of increasing importance. This requires understanding the wider political and institutional environment within which agricultural policy change takes place, and ways in which 'voice', particularly of the poor, can influence change.

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Full Length Research Paper

Semen characteristics of pubertal West Africa dwarf rams fed pineapple waste silage as replacement for dried cassava peel

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In the tropics, ruminants are raised on natural pastures which decline rapidly in quality and quantity during the dry season. This study assessed the reproductive performance of rams fed with cassava–peel–silage (CPS) blended with pineapple-waste (PW) on: scrotal circumference, testicular size and semen volume. Randomized complete block design with four treatments of PW at 0, 20, 40 and 60% w/w in Diets 1, 2, 3 and 4 were used. Each treatment had three replicates while semen was collected once from all replicates in the treatments. Rams were fed experimental diets for 56 days. Cassava-peel was substituted with PW at 0, 20, 40 and 60% w/w in Diets 1, 2, 3 and 4 respectively. Fresh PW were ensiled in plastic mini silos. Samples of freshly processed pineapple waste were taken on the day of ensiling and ensiled products were opened after 21 days to determine their nutritive value. All silage blends had colours from brown to yellow with pH values from 4.0 to 6.8. The scrotal diameter from 16.00cm to 21.40cm and scrotal length from 8.83cm and 12.75cm but did not differ significantly (P>0.05). The sperm motility of rams fed diets 4 and 1 was similar to rams fed other diets. Mean sperm volume, percentage livability and percentage sperm morphology were similar across treatments (P>0.05). It can be concluded that CPS blended with PW up to 60% showed no adverse effect on semen characteristics and fertility of the rams.

Key words: Semen characteristics, pineapple waste, Brewer's dried grain, corn cob, silage.

INTRODUCTION

In Tropical Africa, ruminants are raised on natural pastures which decline rapidly in quality during the dry season and due to urbanization. Changes in nutritional status result in very irregular growth and marked fluctuations in seasonal weights (Wilson, 1987). Small-scale farmers cannot afford the investments required to establish improved pastures and feed concentrate supplements to alleviate dry season growth checks. Therefore, small-scale farmers rely on browse plants, crop residues and by-products to supplement roadside grazing during the dry season. These challenges result in reduction of production of certain livestock species like goats, cattle, swine and poultry. The effects of these challenges have reflected on the quality and amount of animal protein available for human consumption in the third world (Smith et al., 2010). The most crucial constraint affecting livestock production in many developing countries is inadequate animal feed resources. Feed shortages, both quantitative and qualitative, limit livestock productivity. Feed cost is not

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only an important concern in the management of national economies, but, a major cost burden of livestock farms, and thus a major strategy to develop the livestock industry in developing countries could be the use of agricultural by – products like pineapple waste, corn cobs and brewers dry grain (Onwuka et al., 1997). One of such agro-industrial by-products is pineapple waste (PW) from pineapple processing. Pineapple waste occurs as pineapple peels and core, making about 40 to 50% of the fresh fruit (Buckle, 1989) and contains mainly sucrose, fructose, glucose and other nutrients (Krueger et al., 1992). FAO (2004) ranked Nigeria among the leading pineapple producing countries with about 800, 000 metric tonnes, therefore, efforts at finding better use for the PW generated from such huge quantities may be important in terms of environmental pollution and waste as potential animal feed resource. Lamidi et al. (2008) found that broiler chickens could tolerate up to 10% PW in their diets without any deleterious effect. Olosunde (2010) reported that WAD sheep could tolerate up to 45% PW but 30% PW was superior when substituted for corn bran. However, it produced adequate results with older pigs weighing over 57 kg at inclusion rates as high as 50%.

At higher levels, weight gain decreases and feed conversion is depressed. Grinding did not improve daily weight gain and feed efficiency therefore, protein supplementation is required (Göhl, 1982). Due to this high variability, pineapple wastes have been described as equivalent to cereal grains for ruminants (Müller, 1978) or as a low-nutrient feed (Hepton and Hodgson, 2003). In any case, the high amount of fibre makes pineapple wastes more suitable to ruminants than to pigs and poultry. The bulkiness of the fresh products limits intake (O'Donovan, 1978). In adult sheep, the inclusion of up to 14% of dehydrated pineapple waste in Napier grass (*Pennisetum purpureum*) silage did not modify nutrient digestibility but linearly increases dry matter (DM) intake (Ferreira et al., 2009). Dried pineapple could be included at up to 27% in the diet (cottonseed meal and maize grain), replacing fresh Napier grass: the highest DM intake were observed at 11% and the authors recommended 16% as an optimal inclusion rate (Rogerio et al., 2007). Babatunde (1988) classified PW as an alternative feed ingredient to conventional wheat offal. These indicate potential for use as animal feed. The objective of this study is to determine the reproductive potentials of West African Dwarf (WAD) rams fed silage blends of pineapple waste, Brewer's dried grain (BDG) and corn cob, thereby investigating the effects of the silages on the spermatozoa attributes and scrotal characteristics of WAD rams.

MATERIALS AND METHODS

Animals and management

In a randomized complete block design, twelve healthy pubertal WAD rams aged six months were divided into four treatments with three replicates per treatment. The rams were obtained as lambs born to ewes at the Sheep unit of the Teaching and Research Farm of University of Ibadan where the experiment was conducted. The animals were weaned between 63 and 70 days of age with weight ranges between 15.00 and 16.10 kg and housed together in a group in a standard sheep pen with concrete floor till they were six months old. For the period of rearing till six months, they were fed wheat offal based diet concentrate ration supplemented with forage *ad – libitum* and had access to cool clean drinking water at all time. They were allowed out for exercise early in the mornings (0700 h) on days with favourable weather. However, at six months of age, they were subjected to a 56 day growth study to access the effect of pineapple waste silage on their semen after the experimental period. During the trial, animals were given fresh water and salt lick *ad-libitum.* Shown in Table 1 is ingredients (g/100 g) and chemical composition (%) of experimental diets.

Semen collection

Semen was collected once from the rams using electro-ejaculation (EE) method. The electro-ejaculator was used with a rectal probe of about 22 cm long, 2.5 cm in diameter and with two electrodes. The rectal probe was lubricated and gently inserted into rectum, and orientated so that the electrodes were positioned ventrally. The electro-ejaculator was used in automatic setting, applied for few seconds with 2-s rest intervals between stimuli, increasing the voltage stimuli by one volt at a time. The penis was prolapsed beyond the prepuce, and semen was collected into a graduated collection vial and analyzed immediately at room temperature. However, before the collection, the rectum was washed with 6% sodium chloride solution. The probe was then inserted up to about 12 inches and held in a position of rectal floor. Alternate current increasing in voltage gradually from 0 to 5 volts and returning again to zero within every 5 to 10 s was initially passed. The subsequent stimulation made progressively higher so that at about fifth stimulus a maximum of 10-15 volts was reached. Erection and ejaculation was obtained. The source of electric current was AC/220-250 volts/single phase/50 cycles.

Semen evaluation

After collection by electro-ejaculator, the volume of each ejaculate was measured in a graduated tube. The proportion of spermatozoa with an intact apical ridge was evaluated. After fixation in a buffered 2% glutar aldehyde solution and examined under Differential Interference Contrast microscope microscopy at magnification of 400. Total number of spermatozoa per ejaculate was calculated as the product between sperm concentration and volume of the ejaculate. Percentage of abnormal spermatozoa (considering all normal forms in sperm head, intermediate piece and tail) was estimated.

Sperm volume

The volume of the ejaculate was measured with a graduated cylinder. The sample volume can also be determined directly in the collection tube by weighing; assuming 1 ml equals 1 g. Thereby, loss of volume associated with transfer from the collection tube to either another tube or a pipette can be avoided (Jørgensen et al., 1997).

Sperm motility

Sperm motility was assessed by the method described by Zemjanis (1977) and was evaluated microscopically within 2 to 4 min of their isolation from the caudal epididymis and later expressed as

Table 1. Ingredients (g/100 g) and chemical composition (%) of experimental diets.

Ingredient	1	2	3	4
Pineapple waste (PW)	0.00	20.00	40.00	60.00
Dried cassava peel (DCP)	68.00	48.00	28.00	8.00
Brewers dried grain (BDG)	20.00	20.00	20.00	20.00
Ground maize cobs (GMC)	10.00	10.00	10.00	10.00
Urea (U)	2.00	2.00	2.00	2.00
Dry matter	84.57	78.48	68.88	62.59
Crude protein	12.15	12.74	13.42	13.74
Crude fibre	12.44	12.65	13.03	13.68
Ether extract	5.51	4.63	3.75	2.85
Ash	6.06	5.42	4.64	3.58
Nitrogen free extract	63.84	64.56	65.17	66.15
Acid detergent fibre	24.44	26.67	28.58	20.62
Neutral detergent fibre	40.61	43.63	47.20	41.57
Acid detergent lignin	5.46	3.54	3.29	2.45
Hemicellulose	16.17	16.96	18.62	20.95
Cellulose	18.98	23.13	25.29	18.17

percentages. A fixed volume of semen (not more than 10 mL) was delivered onto a clean warm glass slide with a few drops of 2.9% sodium citrate and covered with a 22x22 mm cover slip. The preparation was then examined at a magnification of x400 under a light microscope.

Percentage livability

A drop of semen was mixed with 1% eosin and 5% nigrosine in 3% sodium citrate dehydrates solution for the live/dead ratio as described by Wells and Awa (1977).

Morphology

On a clean, warm glass slide, a drop of semen was placed as well as two drops of Wells and Awa stain. The semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification. The presence of abnormal cells out of at least 400 sperm cells from several fields on the slide was counted and their total percentage estimated (Wells and Awa, 1977).

Statistical analysis

Data obtained were subjected to analysis of variance (SAS, 2000) and where significant difference occurred means were separated using Duncan Multiple range test of the same package.

RESULTS

Sperm motility, percentage livability, sperm volume, mass activity and morphology of rams fed silage blends of pineapple waste, Brewer's dried grain (BDG), cassava peels and maize cob presented in Table 2 are the percentage sperm motility, live/dead ratio, sperm volume, mass activity and morphology of rams fed silage blends of pineapple waste, BDG, cassava peels and maize cob. Scrotal diameter, the mean scrotal diameter in centimeter ranges from 16.00 cm to 21.40 cm. Although, there were variations among the treatments, there was no significant difference (P > 0.05) between groups. Scrotal length, the mean scrotal length in centimeter of the rams fed silage blends varies from 8.83cm in Diet 1 and 12.75 cm in Diet 4. The semen volume ranged between 0.01 ml and 0.23 ml. There was no significant difference (P > 0.05) between the treatments.

Percentage sperm motility ranged between 66.25 and 85.00%. However, this difference was not statistically significant (P>0.05) between groups throughout the experiment. Percentage livability ranged between 87.50 and 90.00. There was no significant difference (P > 0.05) between the treatments throughout the period of the experiment.

The mean mass activity ranged between 3.0 and 3.5. There was no significant between the treatments (P > 0.05) in the sperm mass activity throughout the period of the experiment.

DISCUSSION

There was no significant difference (P < 0.05) in the mass activity of semen across the different stages of the work. This is similar to the effect of the pumpkin plant on the mass activity of semen of WAD bucks as reported by Oyeyemi et al. (2000). There were no significant differences observed in scrotal length and scrotal circumference. However, this work is inconsistent with those obtained by Fernandez et al. (2004), Hotzel et al. (2003) and Oldham et al. (1978) who found that testicular growth can be affected when animals were fed above

Table 2. Scrotal diameter, scrotal length, sperm motility, live/dead ratio, sperm volume, mass activity, colour and morphology of rams fed silage blends of pineapple waste, BDG, cassava peels and corn cob.

Parameter	Treatments			
	1	2	3	4
Scrotal circumference (cm)	20	19.17	16	21.4
Scrotal length (cm)	8.83	12	9.33	12.75
Sperm motility (%)	85.66	83.25	66.25	85
Livability (%)	87.5	90	84.17	87.66
Sperm volume (mL)	0.12	0.1	0.17	0.23
Mass activity	3.33	3.5	3	3.5
Abnormal Morph. (%)	11.84	14.91	12.47	12.96
Colour	Creamy	Creamy	Creamy	Creamy

^{Abc} means in the same row with different superscripts are different at P<0.05.

their maintenance requirement. The number of dead spermatozoa increased but not significantly (P > 0.05) throughout the study. Masters and Fels (1984) reported that testicular size is controlled by nutrition, even to the extent that well-fed rams in spring may have larger testes than poorly-fed rams in autumn. However, the results of the present study depicted that semen volume was not significant (P > 0.05) in treatment group. However, the result of this study did not show any trend in semen volume, therefore semen volume was not significant (P > 0.05) in treatment group. This is contrary to the report of Kheradmand et al. (2006) that there was a tendency for semen volume to increase (P = 0.073) in treatment group, in comparison with the control group. Similarly, this difference was not statistically significant between groups throughout the experiment. Semen volume is one of the important factors in semen evaluation and reproductive performance in the males (Ax et al., 2000).

Although, the differences obtained were not statistically different, semen volume and sperm motility values obtained in this study were respectively lower than the corresponding values reported by Nour et al. (1981) for adult Katjang and Katjang x German Fawn goats. The differences in seminal characteristics could be adduced to breed and species of animals used in these studies. A number of studies have demonstrated that the spermatogenesis in rams is sensitive to increases in protein intake. This effect has been related to an increase in testicular size because it is due to an increase in the volume of seminiferous epithelium and in the diameter of seminiferous tubules, however this result is similar to the report of Hotzel et al. (2003), Abi et al. (1997) and Oldham et al. (1978).

The colour of semen of experimental rams obtained in this study was similar throughout the experiment. This is in accordance to the reports of Bitto et al. (1988) and Oyeyemi et al. (2011), they observed a creamy colour characteristic of WAD buck. In general, total abnormalities per group or as per total sperm cells in all the groups were within normal range and show that increasing plane of pineapple waste did not have any adverse effect on the sperm count. This conflicts the work of Jibril et al. (2011) who found out that feeding high protein diet (17.94% CP) had a negative effect on semen concentration and resulted in lower motility confirming that feeding high level of CP in diet is associated with decline in fertility. Sperm output, sperm morphology, semen volume and sperm viability were not influenced by level and source of protein.

For all semen characteristics studied, there was no significant difference, which agrees with the work of Zeragoza et al. (2009) who observed no difference between feeding levels and semen characteristics in Payoya bucks. Scrotal circumference is an important indicator when observing animals for breeding soundness. It is favorably correlated to testes mass, sperm production, and semen quality, age at puberty, body weight and age in young bulls (Swanepoel and Heyns, 1990). Animals with small testicles have reduced sperm production and poor semen quality, the animals in this study had decreased proportion of functional seminiferous tubules, reduced sperm output and elevated percentage of morphologically abnormal sperm (Cates, 1975; Coulter and Foote, 1979).

Moreover, nutritional factors, more than any others, readily lend themselves to manipulations to ensure positive outcomes (Smith and Akinbamijo, 2000). As indicated by scrotal diameter from this study, testicular size was not affected by the different silage blends. This result supported the hypothesis obtained by Bielli et al. (1999). Lindsay et al. (1984) found no significant effect (P > 0.05) of improved pasture or high dietary protein on testicular dimensions. The volume obtained is similar to that of earlier reports on the WAD bucks by Ajala et al. (2001).

Thompson et al. (1992) reported that scrotal circumference (SC) was not an accurate predictor of sperm morphology or motility when a SC of 32 cm was used to predict the recommended minimal standards for semen quality. In addition, there was no significant linear relationship between SC and either the degree of germinal epithelial loss or the percentage of Grade 4+ seminiferous tubules in the bulls completing the performance test. Knights et al. (1984) reported a favorable genetic relationship of SC with measures of semen quality and quantity. In general, as SC increases in yearling bulls, mass motility, percentage normal sperm, semen volume, sperm concentration and total sperm output increased while the percentage of sperm abnormalities decreased.

Pineapple waste has no adverse effect on the livability of sperm cells. PW can be included in small ruminant nutrition and in sheep production in particular without any detrimental effect on semen characteristic. PW did not reduce fertility in male animals and can be included in the ration of sheep up to 60% without any detrimental effect on the semen characteristics. Therefore, PW can be utilized in WAD ram production to reduce environmental pollution of the wastes which is always disposed indiscriminately.

In conclusion, pineapple waste and cassava peels (CP) compete favorably with conventional protein sources to increase crude protein of WAD rams. Also, feeding 13.74% CP diet formulated using pineapple waste resulted in higher semen quality especially sperm motility, live/dead and sperm volume.

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Full Length Research Paper

Genetic divergence study in improved bread wheat varieties (*Triticum aestivum*)

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The genetic divergence study was conducted to estimate the nature and magnitude of diversity in improved varieties of bread wheat during four Winter season, 2008 to 2012. The divergence analysis including Tocher's, canonical (vector) and Euclidian methods indicated the presence of appreciable amount of genetic diversity in the experimental materials. The thirteen wheat genotypes were grouped into four clusters by both Tocher's and Euclidian methods of divergence study. But the clusters of both methods were different on the basis of the genotypes and their numbers present in the cluster. The result obtained from different methods of divergence study was slightly different from each others. The suitable genotypes for the different characters have been drawn from the all three methods of divergence study. The result of principal component analysis revealed that only three principal component accounted more than 80% of the total variation. 3D diagram based on PCA scores and Euclidian distance matrix reflected highest diversity between PBW343 and HS375 while minimum between RSP564 and RSP561. The information obtained from this study can be used to plan crosses and maximized the use of genetic diversity and expression of heterosis.

Key word: Wheat, genetic divergence, cluster analysis.

INTRODUCTION

The presence of genetic diversity and genetic relationships among genotypes is a prerequisite and paramount important for successful wheat breeding programme. Developing hybrid wheat varieties with desirable traits require a thorough knowledge about the existing genetic variability (Maniee et al., 2009; Kahrizi et al., 2010a, b). The more the genetic diverse parents, the greater the chances of obtaining higher heterotic expression in F1s and broad spectrum of variability in segregating population (Shekhawat et al., 2001). Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for purposeful hybridization (Samsuddin, 1985). The study of genetic divergence can assist in the choice of genotypes to be used in breeding programs for the development of new populations as it estimate the extent of diversity existed among selected genotypes (Mondal, 2003). Several genetic diversity studies have been conducted on different crop species based on quantitative and qualitative traits in order to select genetically distant parents for hybridization (Shekhawat et al., 2001; Arega et al., 2007; Haydar et al., 2007; Ahmadizadeh et al., 2011; Daniel et al., 2011). The present study was undertaken with the aim of examining the magnitude of genetic diversity and characters contributing to genetic diversity among bread wheat

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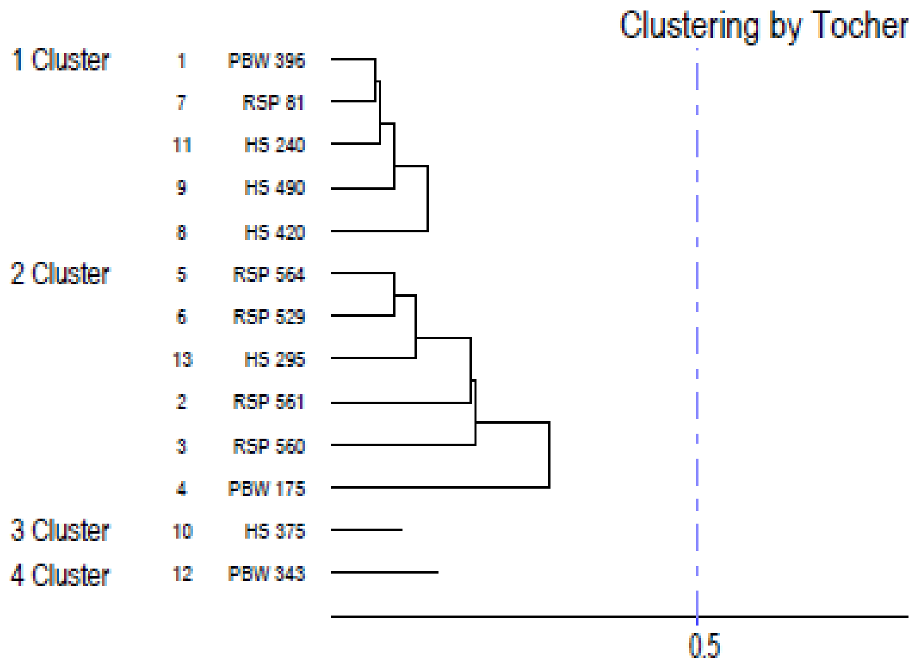

Figure 1. Clustering pattern of wheat varieties by Tocher method.

genotypes for utilization in wheat breeding programme.

MATERIALS AND METHODS

The present experiment was carried out at Maize Breeding Research Station, Poonch (India) under rainfed condition situated between 33° 25' to 34° 01' north latitude and 73° 58' to 74° 35' east longitude at height of 3300 MSL and bounded by Kashmir valley and line of control with Pakistan. The experiment materials consist of 13 bread wheat varieties originated and obtained from different genetical back ground and different centres of the country, respectively (Table 4). All the 13 genotypes are pure line wheat varieties well released for commercial cultivation in different states of India. The experiment was conducted in Randomized Block Design for four years, that is, winter 2008-09, 2009-10, 2010-11 and 2011-12 following the agronomical practice for wheat like row to row distance of 25 cm and fertilizer (NPK) doses at rate of 120:100:80 kg/ha under rainfed condition. The data of physiological characteristics like plant height (cm), spike length (cm) and harvest index (%) were recorded from mean of five randomly selected plants from each variety per replication. days to 50% heading, days to maturity and grain yield were recorded on plot basis where the grain yield was converted into kg/ha. The effective no. of tillers was measured in per square meter of the plot. The experiments were sown on different dates in the month of November during four winter seasons 2008 to 2012. The average rainfall during the crop period (November to May) was 730.5 mm. The genetic divergence among the wheat varieties were calculated by canonical (Vector) and non-hierarchical Euclidean methods of divergence estimation. The D2 values were calculated by using the method described by Mahalanobis (1936). Genetic divergence analysis using canonical (vector) method is a sort of multivariate analysis where canonical vector and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively, were derived (Rao,1952). Non- hierarchical Euclidean cluster analysis (Beale, 1969; Katyal et al., 1985) was conducted using computer package (Windostat version 8.5).

RESULTS AND DISCUSSION

On the basis of results obtained from the present study high degree of genetic divergence was observed. It is evident as more number of cluster (four) formed by the 13 wheat genotypes and high rang of values of inter and intra cluster distance. The more diversity of parents, the greater chance of obtaining high heterosis (Zaman et al., 2005). Amongst four clusters formed by Tocher's method (Figure 1), cluster II was largest (with 6 genotypes) and cluster III and IV were smallest with one genotype only (Table 1). The maximum inter cluster distance was observed between cluster III and cluster IV (Table 2), which exhibited high degree of genetic diversity and thus may be utilized under inter varietal hybridization programme.

The result obtained from different methods of divergence study was slightly differs from each others. Although D2 statistics using Tocher method for classifying the genotypes is useful in general but non-hierarchical Euclidian cluster analysis (based on Wards minimum variance dendrogram) Figure 2 more critically identifies sub clusters of the major groups at different levels and offers additional opportunity than that of Tocher method to plant breeders in planning of hybridization programme aimed at crop improvement.

All the 13 bread wheat genotypes were divided into four clusters by non-hierarchical Euclidian cluster analysis (Figure 2). Maximum number of genotypes (5) grouped in

Table 1. Distribution of 13 wheat genotypes in to four different clusters based on tocher and Euclidean method of cluster analysis.

Cluster	Method	No. of inbred	Name of inbred
I	Tocher	5	PBW 396, RSP 81, HS 240, HS 490, HS 420
	Euclidean	4	PBW 396, RSP 81, HS 375, HS 240
II	Tocher	6	RSP 564, RSP 529, HS 295, RSP 561, RSP 560, PBW 175
	Euclidean	2	RSP 561, RSP 560
III	Tocher	1	HS 375
	Euclidean	5	RSP 564, RSP 529, PBW 175, HS 420, HS 490
IV	Tocher	1	PBW 343
	Euclidean	2	PBW 343, HS 295

Table 2. Mean inter and intra cluster distance among four clusters in wheat genotypes on the basis of D2 statistics (Tocher's Method (I-IV) and Euclidian method (A-D).

Cluster	Cluster I Cluster A	Cluster II Cluster B	Cluster III Cluster C	Cluster IV Cluster D
Cluster I	0.329	0.972	0.633	1.917
Cluster A	3.844	17.161	12.918	21.240
Cluster II		0.700	2.175	1.433
Cluster B		2.858	9.089	15.366
Cluster III			0.00	3.094
Cluster C			5.779	15.896
Cluster IV				0.00
Cluster D				4.513

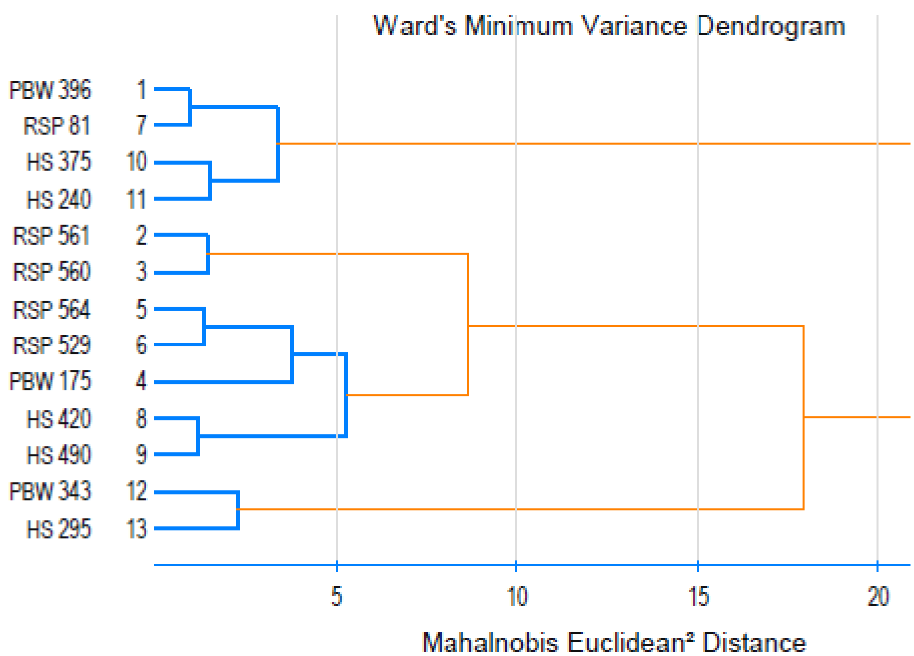

Figure 2. Clustering pattern wheat varieties by Euclidean method.

Table 3. Canonical vectors which supply best linear function of variates, value of canonical roots and percentage of variation absorbed by respective roots.

Parameter	1 Vector	2 Vector	3 Vector	4 Vector
Eigene value (Root)	2.484	1.729	1.504	0.557
% Var. Exp.	35.479	24.694	21.491	7.961
Cum. Var. Exp.	35.479	60.173	81.664	89.625
Days to 50% heading	0.128	0.721	0.087	0.109
Days to maturity	0.395	0.169	0.553	-0.132
Plant height (cm)	0.408	0.255	-0.384	0.607
Effective tillers/ m^2	-0.185	0.003	0.704	0.470
Spike length (cm)	0.546	0.147	0.020	-0.504
Grain yield (kg/ha.)	0.433	-0.397	-0.064	0.357
Harvest index (%)	-0.376	0.455	-0.196	0.006

Table 4. Details of wheat genotypes and their mean values of vectors calculated through canonical (vector) method.

Origin centre	Varieties	Vector 1	Vector 2	Vector 3	Mean yield (Q/ha)
Ludhiana (PAU), India	PBW 396	10.577	15.000	7.342	27.81
Jammu (SKUAST-J), India	RSP 561	10.886	14.301	7.572	38.73**
Jammu (SKUAST-J), India	RSP 560	10.946	14.299	7.599	38.37**
Ludhiana (PAU), India	PBW 175	11.008	14.457	6.624	36.25**
Jammu (SKUAST-J), India	RSP 564	10.906	14.348	7.027	34.73**
Jammu (SKUAST-J), India	RSP 529	10.862	14.219	6.806	32.08**
Jammu (SKUAST-J), India	RSP 81	10.645	15.070	7.277	29.03
New Delhi (IARI), India	HS 420	11.137	14.697	7.057	32.98**
New Delhi (IARI), India	HS 490	10.883	14.868	7.211	34.26**
New Delhi (IARI), India	HS 375	10.773	15.610	7.209	27.54
New Delhi (IARI), India	HS 240	10.921	15.272	7.150	29.89
Ludhiana (PAU), India	PBW 343	9.775	14.256	7.188	27.83
New Delhi (IARI), India	HS 295 (Check)	10.322	14.319	7.016	27.63
CD 1%					4.37

** Significantly different from zero at 0.01 levels of probability.

cluster 3 and minimum (2) genotypes in each of cluster 2 and 4 (Table 1). The relative association among the different genotypes is presented in the form of Wards Minimum Variance Dendrogram which was prepared using the rescaled distance. The resemblance coefficient between two genotypes is the value at which their branches join. The dendrogram elaborate the relative magnitude of resemblance among the genotypes as well as the clusters. It is clear from the perusal of wards minimum variance dendrogram that "fence sitter" single genotype, grouped by Tocher method in cluster III (HS 375) and cluster IV (PBW 343) were precisely accommodated in cluster 1 and II, respectively exhibiting more similarity (less variance) among other members of the respective cluster. Similar type of result was also found by Garg and Gautam (1997) in their experiment.

The result showed that geographical and genetic diversity exhibited no correspondence between them as genotypes from one and different geographic reason are grouped together, which might be due to free exchange of genetic material from different regions. Sharma et al. (2002) and Sharma et al. (2008) have also revealed that the pattern of distribution of genotypes within various clusters was random and independent of geographical isolation. So there is no association between the geographical distribution and genetic diversity. On the basis of Euclidian method the highest inter cluster distance was recorded between cluster 1 cluster 4 consist of 4 and 2 genotypes, respectively, where as minimum between cluster 2 and 3. Within the cluster, the genotypes with high order of divergence were found in clusters 3 followed by cluster 4 (Table 2).

On the basis of cluster mean values, maximum divergence for effective tillers, spike length, grain yield and harvest index was exhibited by cluster 2; days to heading and days to maturity in cluster 1; and maximum divergence for plant height in cluster F (Tables 3 and 4).

Principal factors were carried out using principal

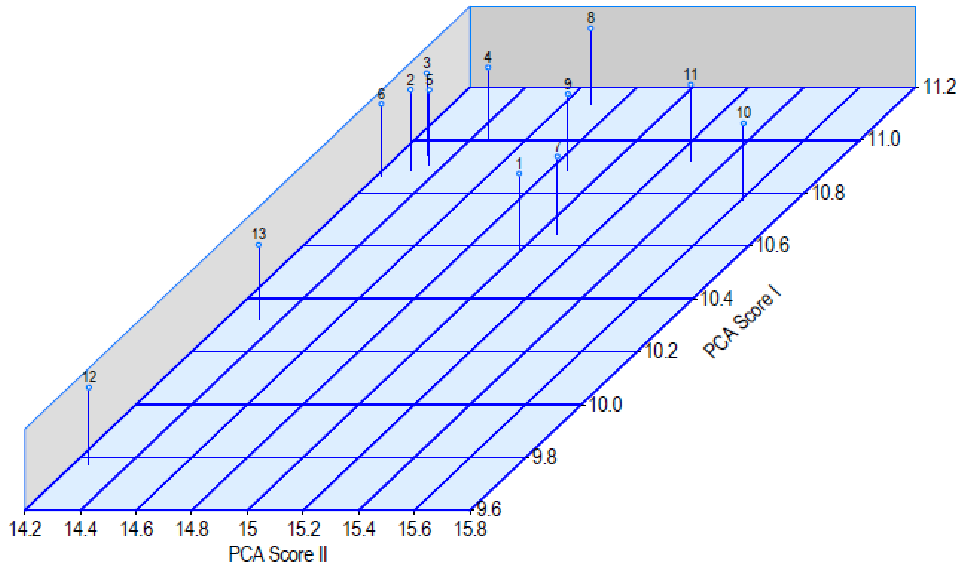

Figure 3. Three dimensional representation of genotypes using 3 principal component based on canonical variates.

component (PC) method for factor extraction. Differentiation among populations occurs in stages, or in other words in different axes of differentiation which accounts for total divergence. Theoretically as many as axes of differentiation can be envisaged as there are characters contributing to total variation, but it is not absolutely. It is possible that most of the variation is accounted for by the first two or more axes of differentiation. In the present investigation only the first three principal components showed eigen values more than one and cumulatively they explained 81.67% variability (Table 3). The first principal component explained 35.48% of the total variation and the second and third principal components explained 24.69 and 21.49% variation, respectively. The first principal component (λ1) absorbed and accounted for maximum (35.48%) proportion of variability and remaining once accounted for progressively lesser and lesser amount of variation for λ2, λ3 and λ4, respectively. The study through canonical analysis revealed that there are three effective axes (vectors) λ1 + λ2 + λ3 = 81.66%. In first axis (vector 1), spike length with element value 0.546; in second axis, days to heading with element value 0.721 and in third axis, effective tillers with element value 0.704, contributed maximum to the total divergence at primary, secondary and tertiary axes of differentiation based on canonical vectors 1, 2 and 3, respectively (Table 4). Similar type of work was also carried out by Tsegaye et al. (2012). Jagadev et al. (1991) reported that the character contributing maximum to the divergence should be given greater emphasis for deciding the type of cluster for purpose of further selection and the choice of parents for hybridization.

Genetic divergence between genotypes is measured in terms of spatial distance and resulted in formation of three dimensional (3D) representation based on three PCA scores (λ1, λ2 and λ3 graphs) as depicted in Figure 3. Three principal factors scores were used to plot all the 13 wheat genotypes using PCA1, PCA2 and PCA3, that is, 3D plot which accounted for most important component traits namely spike length, days to heading and effective tillers.

Amongst 13 wheat genotypes, studied in the present study, exhibited great extent of genetic diversity on the basis of 3D diagram based on PCA scores and Euclidian distance matrix, which reflected highest diversity between PBW 343 and HS 375, while minimum genetic diversity between RSP 564 and RSP 561.

Conclusion

There is significant genetic variability among tested genotypes that indicates the presence of excellent opportunities to bring about improvement through wide hybridization by crossing genotypes with high genetic distance. PCA scores and Euclidian distance matrix reflected highest diversity between PBW343 and HS375 while minimum between RSP564 and RSP561. The information obtained from this study can be used to plan crosses and maximized the use of genetic diversity and expression of heterosis

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Agronomical management influence on the spatiotemporal progress of strawberry dry wilt in Michoacan, Mexico

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The spatiotemporal distribution of strawberry wilt, caused by *Fusarium oxysporum*, *Phytophthora* sp., *Pythium aphanidermatum* and *Rhizoctonia fragariae*, was studied with the aim to establish the effect of some technological components of the strawberry crop cv. Camarosa (*Fragaria* x *ananassa* Duch.) and sustain their use in an integral management of the disease. Epidemics were characterized in two cropping seasons at three localities in Valle de Zamora, Michoacan, Mexico, in commercial plantations with plastic mulch and drip irrigation (A+G), and non-mulch and gravity irrigation (T) on a 100 m^2 area per site. Temporal parameters were contrasting between both management techniques. A+G plantations had significantly lower final incidence (Y_f =12.8±5.6%) than T (22.5±5.9%) (p=0.05) and were consistent with estimators of area of curve (ABCPEa and ABCPEe). The range of epidemic intensity reduction induced by A+G was 22.21 to 76.7% day, which was reflected in lower apparent infection rates (b^{-1}=0.0015-0.0027, R^2=0.92-0.99). Lloyd's Index of Patchiness and Morisita Index (1.01 to 1.17) indicated a slightly aggregated dispersion pattern. Autocorrelation and geostatistical analysis confirmed lower aggregates in A+G (up to 5 plants) vs. T (8 plants), but an apparent higher mobility of inoculum in A+G up to 6.5 m. Plastic mulch and drip irrigation are proposed as technological components of an eventual integrated management program of dry wilt in Michoacan.

Key words: Epidemiology, plastic mulch, drip irrigation, strawberry dry wilt.

INTRODUCTION

Black root rot is a worldwide disease that limits the yield of strawberry and is a serious and common problem that has been reported and studied in mayor producing countries such as Japan, Israel, South Africa, Italy, Spain and the United States (Kohmoto et al., 1981; Yigal et al., 1981; Wing et al., 1994; Botha et al., 2003; Manici et al., 2005; Avilés et al., 2008; Ellis, 2008). Despite its significance, the etiology of black root rot has not yet been fully resolved, and appears to vary according to the site on which it occurs (Botha et al., 2003). In Mexico, strawberry black root rot is commonly known as strawberry dry wilt, caused by the complex *F. oxysporum*, *Phytophthora* sp., *Pythium aphanidermatum* and *Rhizoctonia fragariae* (Ceja-Torres et al., 2008), and reaches incidences of 40 to 80% of the major strawberry producing states in Mexico (Mendoza and Romero, 1989; Castro and Dávalos, 1990). The development of effective strategies for disease management is limited due to the

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success of methyl bromide as a soil sterilant, currently restricted. In addition, the management includes cultivar selection, use of certified planting stock, replacement of plants annually, biological control, rotation crops, soil fumigation prior to planting, soil solarization and use of systemic fungicides during the crop cycle (Yigal et al., 1981; Yuen et al., 1991; Elmer and LaMondia, 1999; Benlioğlu et al., 2005). Moreover, the use of new production technologies such as plastic mulch and drip irrigation influence on the population structure of microorganisms associated with the disease by modifying the soil microenvironment. Therefore, it is important to sustain recent etiological studies of the dry wilt and have a to better knowledge of the development of disease over time and space for identifying options for management of soil-borne diseases (Gilligan, 2002), with additional work to determine the influence of agricultural practices, with emphasis on land cover and irrigation system, and on the spatiotemporal behavior of the disease in order to design management strategies that enhance the possible suppressive effects of conventional crop technologies. This research has been established for this purpose and under the assumption that attributes of intensity of epidemics and pathogen dispersal patterns are strongly influenced by the mulch and the drip irrigation.

MATERIALS AND METHODS

Field experiments

This research was carried out during the 2003-04 and 2004-05 crop cycles in three localities of the Valley of Zamora, Michoacan, Mexico: Ario de Rayón, Tamándaro and Villafuerte. In each locality two commercial plots of 2 ha were chosen for the variety Camarosa; one with plastic mulch and drip irrigation (A+G) and the other with non-mulch soil with gravity irrigation (T). In all cases, the soil was clay. The density was 90 thousand plants per ha set out in zig-zag, double row manner, every 18 cm. The monthly average temperature of the study area was obtained from the Irrigation District 061, Zamora, Michoacan.

Evaluation of the disease

In an area of 100 m^2 (10×10 m) by commercial plot, incidence of wilt plants was recorded monthly from September to January during the crop growth and biweekly from February to May, during flowering and fructification. A plant was considered diseased if it exhibited wilting and gradual death. The spatial location of the plants was recorded using field maps.

Temporal analysis

Epidemics in each of the three regions were characterized by the model of simplified Weibull distribution with two parameters (b and c) (Pennypacker et al., 1980; Thal et al., 1984): $Y = 1-e[-(t/b)^c]$, t>0; where Y = incidence ratio, t = time in days after planting, b = parameter estimator of the epidemic rate in its inverse form, and c = parameter of the curve shape. Additionally, the intensity of epidemics was estimated by calculating the absolute area under the disease progress curve (AUDPCa) by the trapezoidal integration method: $AUDPCa = \Sigma_1^{n-i} [(Y_i + Y_{i+1}) / 2] (t_{i+1} - t_i)$, where: Y_i = proportion of disease in the i-th evaluation, t_i = time at the *i*-th observation, n = number of evaluations (Campbell and Madden, 1990; Jeger and Viljanen-Rollinson, 2001). The parameter AUDPCa) was standardized (AUDPEe) by dividing its value between the time of duration of the epidemics. The relative reduction of area (AUDPEr) in percentage was calculated in relation to AUDPCa of plantations T (AUDPEaT) by location and crop cycle (100-[AUDPCa /AUDPEaT] [100]). Confidence intervals with p = 0.05 and t test were applied for comparison of intensity parameters of epidemics. All data were analyzed with the Statistical Analysis System (SAS) ver. 6.10 (SAS Institute, Cary, NC) (Jesus Junior et al., 2004).

Spatial analysis

The optimum quadrant sizes of (OQS) to calculate indices of aggregation were obtained by the Greig-Smith method (Campbell and Madden, 1990) in blocks 1, 2, 4, 8, 16, 32, 128 and 256 plants with software, MorLloyd version 1.0® MS EXCEL (Rivas and Mora-Aguilera, 2011. Unpublished). The spatial pattern of strawberry dry wilt was determined with Lloyd´s Index of Patchiness (LIP) and Morisita Index (Iδ) (Campbell and Noe, 1985). LIP = m + [(V / m) -1] / m, where m = average number of diseased plants per quadrant, and V = variance. $I\delta = n[\sum(y)^2 - \sum y]/(\sum y)^2 - \sum y$; where: n= total number of quadrants, and y = number of diseased plants per quadrant. Criteria to determine the spatial pattern with these indexes were: 1 = at random, >1 = aggregate and <1 = uniform. The patterns of proximity and spatial dependency of diseased plants were defined by autocorrelation analysis, with the LCOR2® program (Gottwald et al., 1992), and the geostatistical GEO-EAS 1.2.1 software; to determine the spatial dependency in a row (isotropy) and in any direction (anisotropy).

RESULTS AND DISCUSSION

Temporal analysis

Disease onset was delayed until 45 days after planting with an initial incidence less than 1% (Y_0) regardless the agronomic management (Figure 1A to F). However, management significantly influenced the further progress of the disease (Tables 1 and 2). The incidence of the dry wilt increased between 213 and 228 days after planting, being higher in the gravity irrigation system without mulch (T). The flexibility of the Weibull model (Thal et al., 1984) allowed describing the epidemic progress of the strawberry dry wilt with determination coefficients of 0.92 to 0.99 and from 0.97 to 0.99 in the first and second production cycles, respectively. With the exception of Tamándaro and Ario de Rayón in the 2004-05 cycles, the estimator of the epidemic rate (1/b) was lower in A+G. However, only statistical differences (p=0.05) were found in Tamándaro and between this with the T values of Villafuerte and Ario de Rayón (2003-04 cycle) and between Villafuerte (A+G) and Tamándaro (T) (2004-05 cycle) (Table 1). The mechanism of dispersal of the primary inoculum in soil can have large impacts on disease onset, progress, and final incidence (Sujkowski et al., 2000), in this study the shape of the epidemic curve (c) in all cases was sigmoidal asymptotic, typical of

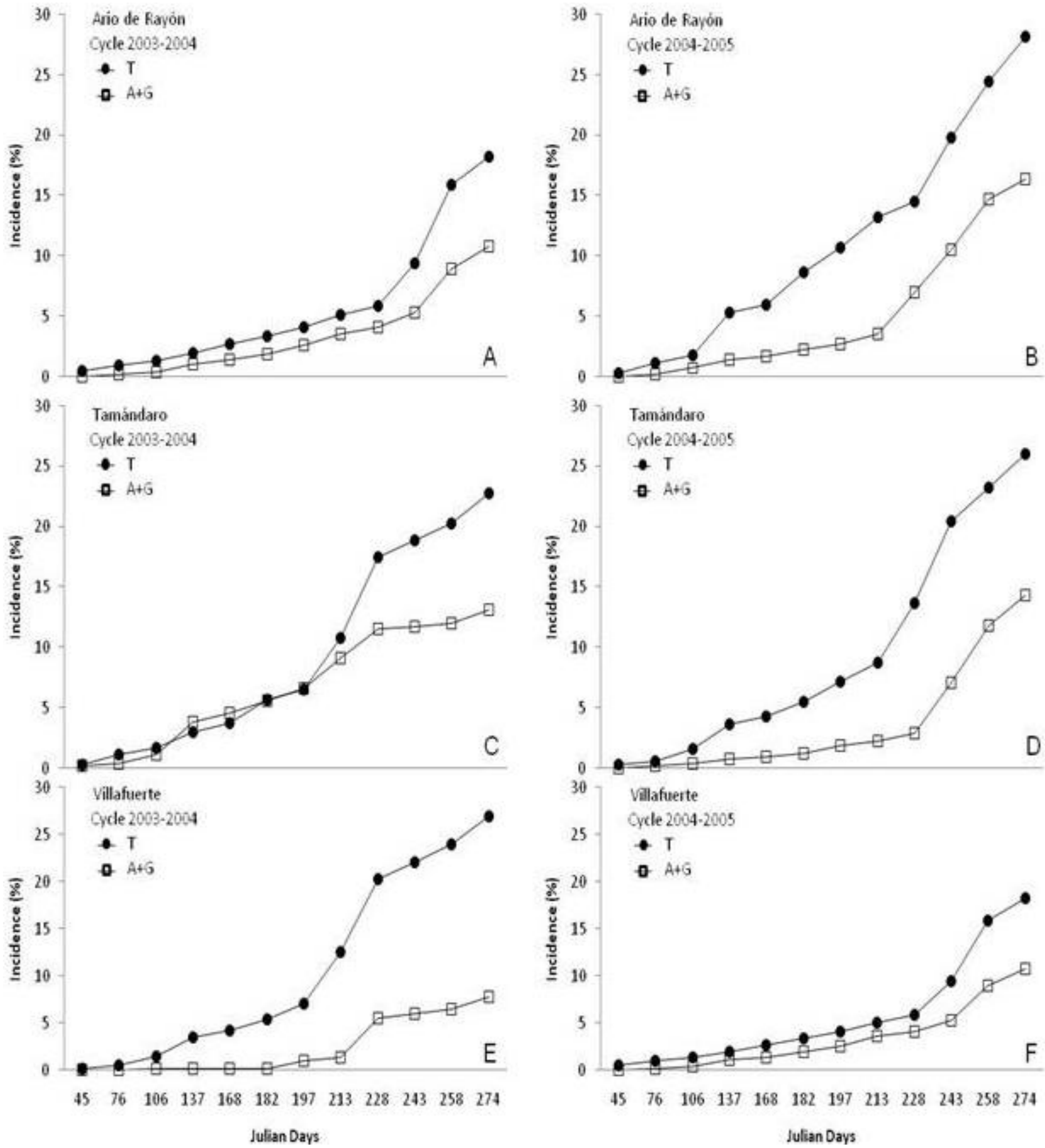

Figure 1. Curves of the temporal progress accumulated of strawberry dry wilt in plantations with plastic mulch and drip irrigation (A+G) and non-mulch and gravity irrigation (T), in the Valley of Zamora, Michoacan, Mexico (A, C and E 2003-04 cycle; B, D and F 2004-05 cycle).

low-level epidemics with limited inoculum dispersal. In this case, statistical differences were also detected between the curve shapes being clearer between Tamándaro (A+G) and the other localities in both cycles. Although the intensity of disease was generally lower in A+G, Weibull analytical results were not fully consistent with the graphical inspection of the curves where less epidemic intensity was detected in A+G (Figure 1B to F). Final incidence (Y_f) in the 2003-04 cycle ranged from 7.8 to 14.5% (11.8 ± 3.5%) in plantations A+G and 13.1 to 27% (21.7 ± 7.1%) in T, and in the 2004-05 cycle, 10.8 to 16.4% (13.8 ± 2.8) and from 18.2 to 28.2% (24.1 ± 5.3%) in the same order (Table 2). The significance of mean differences in the first and second cycles was 11.6 and

Table 1. Description of 12 epidemics of strawberry dry wilt by the Weilbull model for the 2003-04 and 2004-05 cycles in the Valley of Zamora, Michoacan, Mexico.

Locality (management)	Model $Y= 1-e[-(t/b)^c]$	R^2	Interval of confidence (95%)*	
			b	c
2003-2004 cycle				
Ario de Rayón (A+G)	$Y= 1-e[(t/426.6)^{3.99}]$	0.97	381-472[a]	3.22-4.77[a]
Ario de Rayón (T)	$Y= 1-e[(t/410.4)^{4.84}]$	0.99	395-426[a]	4.47-5.21[a]
Tamándaro (A+G)	$Y= 1-e[(t/653.0)^{2.16}]$	0.97	516-790[b]	1.72-2.60[b]
Tamándaro (T)	$Y= 1-e[(t/397.0)^{3.41}]$	0.96	352-442[a]	2.64-4.19[a]
Villafuerte(A+G)	$Y= 1-e[(t/459.0)^{4.69}]$	0.92	361-558[ab]	3.01-6.37[a]
Villafuerte (T)	$Y= 1-e[(t/366.1)^{3.67}]$	0.96	331-402[a]	2.82-4.51[a]
2004-2005 cycle				
Ario de Rayón (A+G)	$Y= 1-e[(t/374.6)^{5.26}]$	0.98	348-401[ab]	4.31-6.21[bc]
Ario de Rayón (T)	$Y= 1-e[(t/386.9)^{3.24}]$	0.99	305-409[ab]	2.86-3.61[a]
Tamándaro (A+G)	$Y= 1-e[(t/355.0)^{6.99}]$	0.97	323-378[a]	5.59-8.39[c]
Tamándaro (T)	$Y= 1-e[(t/362.6)^{4.07}]$	0.98	338-387[a]	3.38-4.75[ab]
Villafuerte(A+G)	$Y= 1-e[(t/435.8)^{4.68}]$	0.98	397-475[b]	3.92-5.44[b]
Villafuerte (T)	$Y= 1-e[(t/375.1)^{5.06}]$	0.97	341-409[ab]	3.91-6.20[bc]

b, Estimator of the apparent infection rate in its inverse form (1/b); c, estimator of the curve shape. *Values of same parameter for each crop cycle, with different letter are statistically different (p = 0.05%).

Table 2. Absolute, relative and standardized AUDPE, Y_f y b^{-1} (Weibull) of 12 cumulative progress curves of strawberry dry wilt for the 2003-04 and 2004-05 cycles in the Valley of Zamora, Michoacan, Mexico.

Locality	Management	AUDPEa	AUDPEr	AUDPEe	Y_f	b^{-1}
2003-2004 cycle						
Ario de Rayón	A+G	7.58	-30.69	0.033	14.5	0.0023
Ario de Rayón	T	5.80	0.00	0.025	13.1	0.0024
Tamándaro	A+G	11.35	22.21	0.050	13.1	0.0015
Tamándaro	T	14.59	0.00	0.064	22.8	0.0025
Villafuerte	A+G	3.84	76.63	0.028	7.8	0.0022
Villafuerte	T	16.43	0.00	0.072	27.0	0.0027
2004-2005 cycle						
Ario de Rayón	A+G	8.59	57.14	0.038	16.4	0.0027
Ario de Rayón	T	20.04	0.00	0.088	28.2	0.0026
Tamándaro	A+G	5.89	64.96	0.026	14.3	0.0028
Tamándaro	T	16.81	0.00	0.073	26.0	0.0028
Villafuerte	A+G	5.74	42.94	0.025	10.8	0.0023
Villafuerte	T	10.06	0.00	0.044	18.2	0.0027

A+G, Plastic mulch with drip irrigation; T, non-mulch and gravity irrigation.

0.05%, respectively. Weibull failure to reflect these trends could be due to the extension of the lower asymptote which was due to the delay that A+G caused in the increase of the epidemics. Another alternative analytical unaffected by the asymptotic factor was AUDPE. Lower values of AUDPEa and AUDPEe were obtained with the operation A+G except in the 2003-04 cycle in Ario de Rayón which was consistent with the values of Y_f (Table 2). The Weibull rate parameter (b^{-1}) was significantly correlated with AUDPEa but with low accuracy (r^2=0.58) confirming its limited ability to describe in the context of this work. The reduction range of epidemic intensity of A+G on T was 22.1 to 76.6% and 42.1 to 64.9% in 2003-04 and 2004-05 cycles, respectively, which demonstrates the strong suppressive effect of the combination of plastic mulch with drip irrigation (Table 2). There were no

Table 3. Spatial pattern of strawberry dry wilt with different crop management for the 2003-04 and 2004-05 cycles in the Valley of Zamora, Michoacan, Mexico.

Locality (management)	Optimum quadrant size	LIP	Morisita Index	Spatial pattern
2003-04 cycle				
Ario de Rayón (A+G)	128	1.09	1.07	Aggregate
Ario de Rayón (T)	128	1.02	1.01	Aggregate
Tamándaro (A+G)	128	1.02	1.02	Aggregate
Tamándaro (T)	128	0.99	0.99	Uniform
Villafuerte (A+G)	128	1.14	1.12	Aggregate
Villafuerte (T)	128	1.07	1.05	Aggregate
2004-05 cycle				
Ario de Rayón (A+G)	32	0.94	0.96	Uniform
Ario de Rayón (T)	32	1.09	1.06	Aggregate
Tamándaro (A+G)	32	1.17	1.10	Aggregate
Tamándaro (T)	32	0.98	0.98	Uniform
Villafuerte (A+G)	16	1.01	1.01	Aggregate
Villafuerte (T)	32	1.05	1.04	Aggregate

differences between cycles with no parameters (p = 0.05) suggesting that the increase of inoculum required longer periods of time, typical of soil organisms (Michreff et al., 2005).

The temporal analysis of dry wilt showed that the A+G management affected the efficiency of fungal and pseudofungi inoculum associated with the disease, leading to a lower intensity of epidemics, but not total elimination capacity. The increase in incidence in all cases coincided generally with fructification and average room temperature during this period between 19 and 25.5°C, range biasing the expression of pathogens associated with diseases of root diseases in part by the increase of the transpiration rate combined with the productive stress of the plants (Michereff et al., 2005). In Australia *Fusarium oxysporum* and binucleate *Rhizoctonia* particulary AG-A, caused severe disease on root and crowns, resulting in the eventual death of plants, still severely retarded the growth and development at 27°C, but *Macrophomina phaseolina* was most virulent and caused most severe disease at 32°C (Xiangling et al., 2011), this last pathogen has not been reported in Mexico. Although soil temperature was not measured, plastic mulch can increase temperature from 3 to 7°C and drastically change the soil moisture (Mbagwu, 1991; Schmidt and Worthington, 1998), which could explain the reduced efficiency of inoculum in the initial phase of the epidemics, as evidenced by the prolongation of the initial asymptote A+G. It is shown that these factors differentially affect the pathogenicity of microorganisms (Pinkerton et al., 2002; Michereff et al., 2005). For example, *Phytophthora capsici* was more aggressive in *Capsicum annuum* and caused a higher incidence of wilting than *Rhizoctonia solani* due to soil temperature (20 to 22°C) and humidity at field capacity not optimal for this fungus (Vázquez-López et al., 2009). Previous studies with strawberry dry wilt indicate that this disease increases in poorly drained clay soils (Castro and Dávalos, 1990; Mendoza, 1992), which was characteristic of plots under study. In a subsequent regional study it was confirmed that the distribution and prevalence of fungi and pseudofungi causing of strawberry dry wilt was influenced by soil texture and other factors such as the level of organic matter (Ceja-Torres et al., 2008).

Spatial analysis

Optimum quadrant size (TOC) for use in the calculation of Lloyd's Index of Patchiness (LIP) and Morisita Index (Iδ) was 120 plants in 2003-04 and 32 plants in 2004-05 from a matrix of data of 40 × 20 in the first crop cycle and 20 × 20 in the second crop cycle (Table 3). Exploratory maps were obtained using SURFER 4.0® (Figure 2) and the LIP indices of 1.01 to 1.17 and Iδ of 1.01 to 1.12, indicated that dry wilt of strawberry had a pattern of slightly aggregated dispersion in 75% of the plantations studied predominantly at A+G (83.3%) (Table 3). This is because the values of these indices were slightly higher than one, indicating weak aggregation, which verifies previous observations regarding the distribution of the disease in patches (Téliz et al., 1986). Only two plantations with gravity irrigation and one with drip irrigation, showed a trend toward a uniform pattern of disease (LIP and Iδ of 0.94 to 0.99) (Table 3). These results suggest that the distribution pattern of the inoculum is influenced by the plantations management in addition to biological attributes inherent in the aggregate behavior of some pathogens due to the effect of rhizosphere (Mora-Aguilera et al., 1990) and to

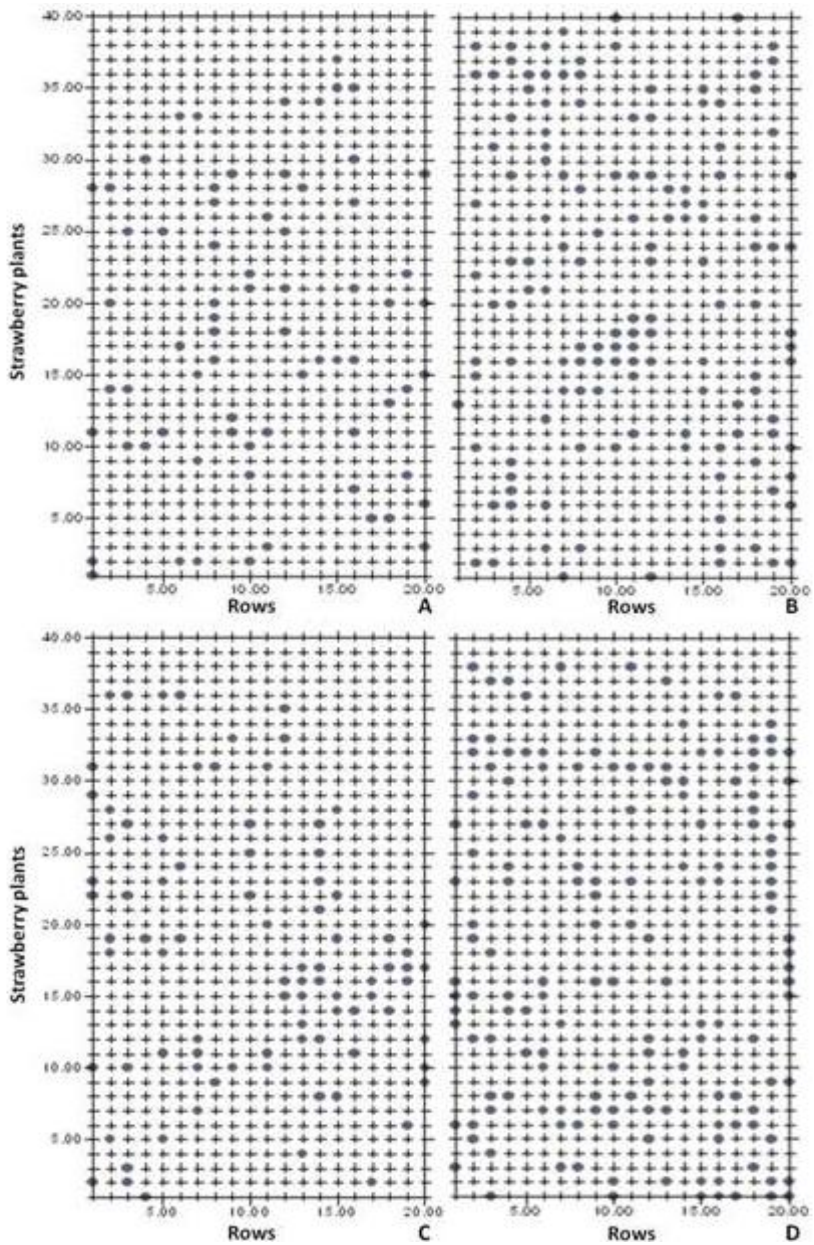

Figure 2. Spatial behavior of strawberry dry wilt into quadrants of 100 m^2 (10 × 10 m) in Tamándaro (A and B 2003-04 cycle, C and D 2004-05 cycle). Plantations with plastic mulch and drip irrigation (A+G) left, and non-mulch and gravity irrigation (T) right.

competition for sites of infection between individuals which can result in consistent patterns of damage (Ludwig and Reynolds, 1988).

Autocorrelation analysis confirmed the spatial effect of management and allowed to estimate attributes of distance and directionality of dispersal. The greatest dispersion in aggregates (continuous dependence) and sub-aggregates (discontinuous dependence), it is generally found in Ario de Rayón and Tamándaro in both crop cycles. In Villafuerte only sub-aggregates were found (Table 4).

Confirmation of aggregates within and between rows was of order 1, with exception of Tamándaro with order 2, which implied small patches of diseased plants. The sub-aggregates were in the range of order 2 to 20 with higher dominance within the row. The epidemic intensity level was not associated with a specific spatial pattern possibly by Y_f less than 23% (Table 2 and Figure 1). With regard to the management, higher aggregation was found in T plantations; with patterns of 1 to 5 diseased plants, by

Table 4. Spatial self-correlation to determine spatial dependence of strawberry dry wilt with two irrigation technologies and three locations of Valle de Zamora, Michoacan, Mexico.

Location (management)	Spatial dependence		
	Within rows	Between rows	GG
2003-04 cycle			
Ario de Rayón (A+G)	C1**, D5*, 11** and 20*	C1**	+
Ario de Rayón (T)	C1*, D7** and 15**	-	+
Tamandaro (A+G)	D8* and 14**	D6*	+
Tamandaro (T)	C2*, D19*	C1**, D13* and 16*	+
Villafuerte (A+G)	D3*	D10**	+
Villafuerte (T)	D2*, 7* and 15**	D2*	-
2004-05 cycle			
Ario de Rayón (A+G)	C1*, D7* and 27*	-	+
Ario de Rayón (T)	D2*, 3**, 4**, 5** and 14*	C1**, D7*, 8**, 15** and 17**	+
Tamandaro (A+G)	C1**, D16**	D2**, 4*	-
Tamandaro (T)	C1**	C1**, D3**, 7** and 10*	+
Villafuerte (A+G)	D8** and 18*	D2* and 12*	-
Villafuerte (T)	D12**	D2* and 7**	+

GG, Continuous general gradient (aggregated); C, continuous dependence; D, discontinuous dependence. The number indicates the "order" of the dependence.

autocorrelation and 1 to 8 plants with geostatistics while in A+G were 1 to 3 and 1 to 5 plants by the respective analysis, which made it possible to visualize patterns of spatial dependence in different directions (Nelson et al., 1999). The dominant sub-aggregates were formed up to 4.5 and 6.5 m in plots T and A+G, respectively. Remarkably, A+G had the smallest aggregates, but the restrictive space could explain the highest displacement of the inoculum within the row. This level of dispersal is consistent with root pathogens, because it depends on the spatial pattern of host population, especially when disease transmission requires contact between healthy and susceptible tissues, that is, root to root (Sujkowski et al., 2000; Willocquet et al., 2000) and the airborne pathogens have a less restrictive dispersion. Since 2006, *Fusarium* wilt of strawberry has increased in incidence and severity in California, USA. Initial problems in 2006 consisted of multiple small patches (2 to 4 beds wide × 3 to 10 m long) of diseased plants; in these patches disease incidence could range from 80 to 100%. By 2009, in some fields, the disease affected large sections that ran the length of the field (Koike et al., 2009). Moreover, the existence of spatial dependencies presupposes the action of a common inoculum source (primary source of infection). This may be valid for a single pathogen-host association. Strawberry dry wilt is caused by a complex of pathogens which involves various sources of inoculum and roles of primary and secondary infection, originating from soil-borne inoculum and diseased plants, respectively (Willocquet et al., 2007; Ellis, 2008; Xiangling et al., 2011). Hence the interpretation of spatial dependencies cannot be via a conventional method without considering other effects such as competition and aggression.

Conclusions

The commercial applications of plastic mulch and drip irrigation (A+G) for the purpose of productivity of strawberry crops in the Zamora Valley were effective in reducing inoculum potential of organisms associated with dry wilt, although these did not induce a total suppressive action. Spatiotemporal studies in two production cycles and three localities selected for high inductance to the disease confirmed in all parameters used (yf, AUDPCa, AUDPEr, AUDPEe, b^{-1}) the lowest epidemic intensity in plantations A+G with reductions in the range of 22.2 to 76.6%. Similarly, the indexes and spatial statistical analysis showed lower aggregates in A+G (up to 5 plants) but found an apparent increased dispersiveness along the row, possibly as a result of its plastic enclosure (up to 6.5 m). The plastic mulch and drip irrigation should be adopted in a program of integrated management of dry wilt. Future studies may be aimed to optimize these technologies with respect to the suppressive ability of the organisms associated with the disease.

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Full Length Research Paper

Influence of leaf extract of *Jatropha curcas* on initial growth of cauliflower (*Brassica oleracea* var. botrytis)

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This paper aimed at evaluating the initial growth of cauliflower (*Brassica oleracea* var. Botrytis) subjected to various levels of aqueous extract of leaves of Jatropha (*Jatropha curcas*) according to the following parameters: stem diameter, stem length, root length, leaf length and leaf area, root dry mass, leaf dry mass, root fresh mass, and leaves fresh mass. The experimental design was randomly blocks with six concentrations (0, 5, 10, 20, 40, and 80%) and six replications. The study was conducted at the experimental campus of the State University of West Paraná, UNIOESTE, campus Cascavel. The statistical analysis of the results showed a significant effect of concentrations of aqueous extract of *J. curcas* on the initial growth of cauliflower. Except for root growth, there was a positive effect dependent on the increased concentration of crude aqueous extract.

Key words: Cauliflower, jatropha, allelopathy.

INTRODUCTION

Cauliflower is an inflorescence-type vegetable that belongs to the species *Brassica oleracea* var. botrytis L., whose delicate and tender texture requires care and attention in its preparation (Filgueira, 2000). It is cultivated in the Middle East since antiquity. However, only from the twelfth century this culture began to expand to other parts of the world, being grown in Brazil in the States of São Paulo, Rio de Janeiro, Rio Grande do Sul, Minas Gerais, Paraná, and Santa Catarina. In Paraná, it is among the leading vegetables produced in the Metropolitan Region of Curitiba (Hasse, 2005; May et al., 2007).

China is the country with the title of world's largest producer of cauliflower, followed by India, Spain, Italy, and France (FAO, 2009). It is a vegetable containing appreciable amount of vitamins, free of fats and cholesterol and with low levels of sodium and calories (May et al., 2007).

According to Filgueira (2008), with the development of genetic studies, there was the development of hybrid cauliflower which has suitable production conditions in hotter climates, a factor that promoted the cultivation throughout the year, since it is a plant which generally requires low temperatures for cultivation (Blanco et al., 1997). With regard to Jatropha (*Jatropha curcas* L.), it is a perennial and monoecious species, belonging to the family Euphorbiaceae, the same of castor (*Ricinus* sp.), cassava (*Manhiot* sp.) and rubber (*Hevea* spp.). It is believed that, Jatropha is a native of Central America; however spontaneous vegetation in various regions of Brazil (Heller, 1996; Beltrão, 2005). Its leaves have high amount of nutrients which when extracted, can serve as a substrate for other cultures. Nutrients accumulated by the leaves include: N, Ca, K, Mg, P, S, Mn, Fe, B, Zn, and Cu.

Previous studies have shown that, the root and stem

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extracts of *J. curcas* have allelopathic effects on some crop species (Abugre et al., 2011; Rejila and Vijayakumar, 2011), also being observed phytotoxicity of its residues in soil (Wang et al., 2009). Igbnosa et al. (2009) suggests that, leaves of *J. curcas* exert greater allelopathic effect on germination, length of radical, and plumule of plants. It also states that, higher amounts of allelochemicals can be found in leaves than in roots of *J. curcas*. This is corroborated by the findings of Maharjan et al. (2007), where the preliminary screening showed that, the leaf extract had the strongest allelopathic effect on seed germination. Tefera (2002) also found that, the impact of allelopathic leaf extract was more powerful than other vegetative parts.

With respect to the effects of a plant (or microorganisms) on another plant, defined the term allelopathy from the Greek: allelon means from one to another and pathós means suffering, as the chemical interaction that occurs between organisms from the release of substances derived from the secondary metabolism of plants, so-called allelochemicals. According to the IAS (2010), this process can be defined as the studies involving any process with secondary metabolites which are produced by plants, algae, bacteria or fungi, which may affect the growth and development of agricultural production and biological systems. When released, the allelochemicals may influence the growth and development of surrounding biological systems (Razavi, 2011) and can even be used to control undesirable plants (Appleton and Berrier, 2009) in agriculture, in rotating systems such as *Helianthus annuus* that when grown before soybean (*Glycine max* (L.) Merr.) reduces the amount of weed species (Pasqualeto et al., 2007), terpenes, and phenols (Corsato et al., 2010) that can combat herbivorous, insects, and fungi, besides influencing the growth of other plants (Taiz and Zeiger, 2009).

Regarding bioassays, these procedures are used to evaluate the allelopathic potential of a given species through global parameters such as germination, growth, and development of seedlings or adult plants (Souza-Filho and Alves, 2002), where tests that add experimental procedures that characterize the allelopathic property of certain crops serve to contribute to the studies of dynamics between species, development of management and production strategies (Souza-Filho et al., 2010).

Therefore, considering the above, this study aimed at determining the effects of irrigating cauliflower crop using leaf extract of *J. curcas* in different proportions, as well as evaluating the development of the plant according to the following parameters: stem diameter, stem length, root length, leaf length and leaf area, root dry mass, leaf dry mass, root fresh mass, and leaf fresh mass.

MATERIALS AND METHODS

This work was carried out during the autumn of the year 2013 at the State University of West Paraná, UNIOESTE campus Cascavel, PR, Brazil. The local climate is considered subtropical mesothermal and super humid with mean annual temperature of 19°C, mean rainfall of 60 mm per month distributed over all months of the year (Brazil, 1999). Before mounting and evaluation of experiments, the bench, hands and materials were disinfected using bactericide solution (Lysoform) and fungicide (Nystatin), both at 10%. Seeds of cauliflower were acquired in suitable trade.

To carry out this research expanded polystyrene trays with 200 cells was used, with a 10 × 20 cell division, each with 14 cm^2 and 5.5 cm deep.

Extraction and extract preparation

As a source of irrigation was employed, the use of leaf extract of *J. curcas* from the experimental field of UNIOESTE campus Cascavel. 400 g of leaves were crushed in 2 L of distilled water using a blender. Then, suspensions were prepared containing specific amounts (weight g) of crushed plant species and a specific volume (ml) of distilled water according to the desired concentration, which are: 0, 5, 10, 20, 40, and 80% dilution. The solution was filtered through four layers of gauze at 15 × 15 cm^2. Subsequently, the extracts were stored in specific sprayers and packed under refrigeration at 10°C throughout the experimental period in order to preserve their characteristics.

Planting of seeds and extract application

Each cell was filled with commercial extract and two seeds were inserted at 1 cm depth of each cell. The treatment was performed as follows: thirteen lines were used, the first boundary line (untreated), followed by the treatment with line 0% of extract (control), boundary line, line with 5% of extract, boundary line, line with 10% of extract, boundary line, line with 20% of extract, boundary line, line with 40% of extract, boundary line, line with 80% of extract followed by the final line of boundary.

Sowing occurred on the 28 of March, 2013. The treatment was performed from the first day of sowing, occurring in random blocks with 10 cells for each treatment. Six extract doses with the aforementioned dilutions were evaluated, and each dose was administered in three equal portions a day, totaling 100 ml of substrate by treatment.day^{-1}. After four weeks of planting, the harvesting of plants, preserving the root of each one was carried out. The root systems were air dried and weighed. To a caliper Tramontina brand was used to measure the stem diameter, stem length, root length, and leaf length. In determining the leaf area by point's method, transparency sheets containing digitized points with graph paper in 1 cm^2 square equispaced each was used, and were then counted points met by the contour of each sheet. Thus, the leaf area was estimated by the number of points completed (Peixoto and Peixoto, 2009). For analyses of fresh and dry mass of leaves and roots, paper envelopes of 10 cm^2 were used, where leaves and roots of 6 plants of each treatment were inserted, separately. The envelopes were weighed on analytical balance, Marte, AY220 model, year of manufacture 2011 and taken to oven at 65°C for 24 h, which were subsequently weighed again.

Statistical analysis

Data analysis was performed using the software ORIGIN 8.5, producing graphics and fit of curves. Student's t test was used, the software version 7.6 beta ASSISTAT from the regression analysis at 5% probability of error.

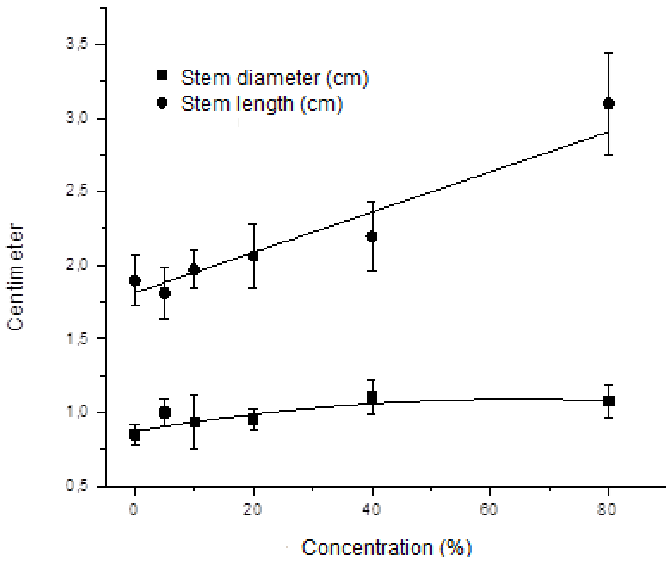

Figure 1. Variation of parameters: Stem diameter (▪), stem length (●), with different extract levels.

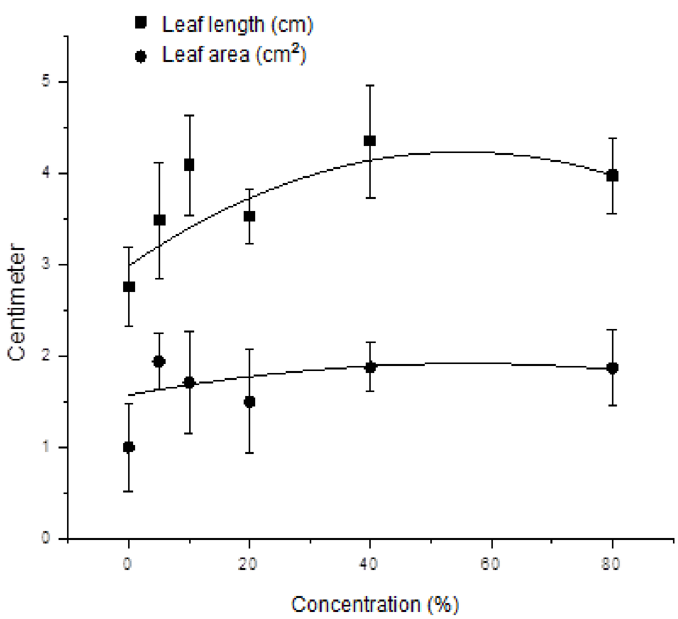

Figure 2. Variation of parameters: leaf length (▪), leaf area (●), with different extract levels.

RESULTS

Figure 1 shows that, the effect on growth of stem diameter by the extract of *J. curcas* does not have a linear behavior. Although the F-test analysis with 5% significance has provided that the control value is different from those obtained with the treatments of 5, 20, 40, and 80% extract, the level of 10% extract was found to be not significant due to its high standard deviation value.

Figure 1 also shows the effect of the extract on the stem length, the linear fit whose Pearson coefficient is 0.899, the line equation obtained is Y = 1.814 + 0.013 X. According to the values obtained, the extract concentration of 80% caused on average of 1.3 cm increase compared to the control sample. With respect to leaf length (Figure 2), its behavior was growing as the level of *J. curcas* extract was increased. The t test indicated difference values between the last two samples and control with 0.05 significance. In the same figure we have the behavior of leaf area with increasing extract concentration. According to the observed values, there is increased mean value of leaf area from 1.0 to 1.86 cm^2 (86% higher). The t test indicated a significant difference between the last two samples and control at 0.05 significance.

Figure 3 shows that, the treatment with different concentrations of extract leads to a significant increase in the fresh mass of leaves and roots. The fitted lines were Y = 0.3712 + 0.0046 X to the leaves and Y = 0.0820 + 0.0010 X to the roots. According to these settings there is 12.4% increase in the mass of leaves and 12.1% for mass of roots for each 10% increase of extract used.

Figure 4 shows a linear increase of dry mass of leaves and roots up to 40% extract of *J. curcas* used. The point in 80% had no proportional increased to the previous points, indicating a tendency to stabilize its value at the extract concentration of 80%. Using the values, up to 40% of extract was obtained the line equation of Y = 0.0282 +0.00039 X to the leaf (DL) and Y = 0.0101 +0.00012 X to the root (DR). The Pearson coefficients were greater than 0.96 for treatments used.

By substituting the mass value obtained at 80% in the line, equation is obtained that the extract concentration required to produce the greatest gain would be 46.4% to leaves and 49.2% to the root, that is, extracts around 50% would cause approximately 64.7% increase in the mass of leaves and about 66.7% increase in the mass of roots compared to the mass in the control.

For root length, the values of this parameter do not differ markedly from each other. The Student's analysis indicated that, means with 5, 10, 20, and 80% extract did not differ from control sample and, the sample with 40% extract of *J. curcas* has a very close value to the limit to accept the null hypothesis. Considering the result, the graph for root length was not exposed. Data with regard to this parameter can be found in the Table 1.

Table 1 shows in summary the means results and their standard deviations for the parameters studied and the percentage of variation for samples of 40 and 80% compared to the control sample. Table 1 summarizes the results obtained using treatment with the extract of *J. curcas*. In general, there was increase in the diameter values of the stem, stem length, leaf length, and leaf area as the extract concentration also increased. The t test at

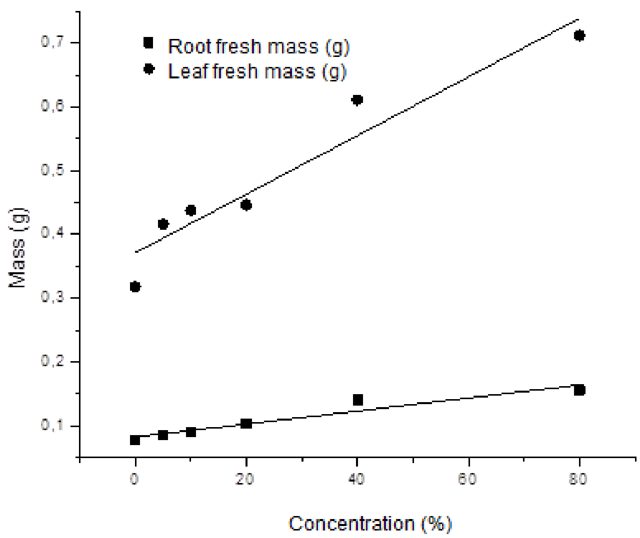

Figure 3. Variation of parameters: root fresh mass (▪); leaf fresh mass (●), with different extract levels.

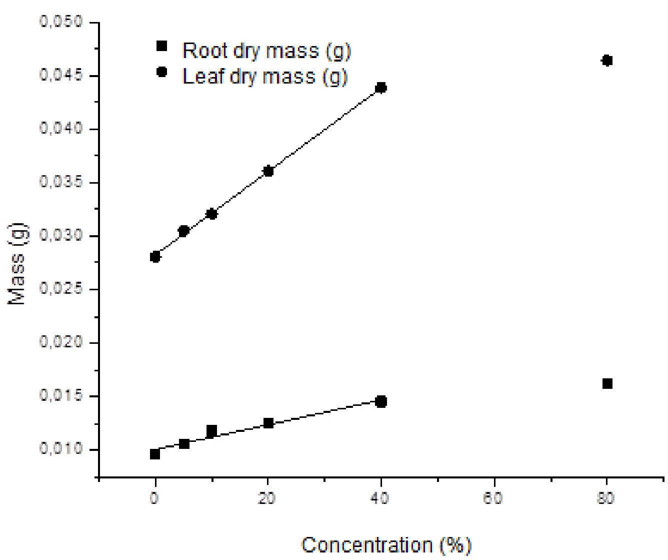

Figure 4. Variation of parameters: root dry mass (▪), leaf dry mass (●), with different extract levels.

0.05 significance level confirmed that the control value (concentration 0%) differs from the values obtained with 40 and 80% of extract for quantities mentioned in the previous paragraph. Only the variation in root length was not significant according to the t test. Increases compared to control (in percentage) are presented in the last two rows of Table 1, being possible to verify a sharp increase of leaves characteristics, increasing their area by 87% approximately. Significant values were also observed for stem diameter and stem length, but less pronounced as those found for the leaf.

DISCUSSION

The stem development in the parameters length and diameter (Figure 1) found in this work differs from the results of Abugre and Sam (2010) who suggest that, the growth inhibition of seedlings of *Zea mays* exposed to high concentrations of root extract of *J. curcas*. In this work, the extracts of *J. curcas* provided positive development of cauliflower.

Despite their having been no increase in root length, mass gain was achieved, indicating a higher number of roots and in some cases, increase of its diameter. Bonamigo et al. (2009) found a positive effect of the application of aqueous extract of *J. curcas* for the mean root length of the seedlings of *Brassica napus*. These contradictory results are possibly related to the allelopathic properties of the extract of *J. curcas*.

The increased leaf length (Figure 2) obtained in this study does not agree with the results of Wang et al. (2009). The authors reported allelopathic effect of leaves of *J. curcas* in seedling development of *Tagetes erecta* L. According to the authors, the leaves caused decreased shoot length of the seedlings evaluated and when added the residues of *J. curcas* on the ground, there was increased toxicity, exerting greater phytotoxicity in seedlings root system. Regarding the increased leaf area found in this study (Figure 2), data diverge from those of Rejila and Vijayakumar (2011), where the aqueous extracts of *J.curcas* showed inhibitory effects on shoots of *Capsicum annum* L. (green pepper), this increasing effect with the increased levels of substrate. Similar effects to those in the work of Rajangam (1984), where the leaf area development of *Oryza sativa* and *Z. mays* were inhibited by *Rhizophora apiculata*, and in peanuts by bamboo (Eyini and Jayakumar, 1989). Data corroborate Palani and Dasthagir (1998), who reported leaf area reduction of cultivated peanut by aqueous extracts of leaves of *Eucalyptus globulus*.

The observed increase in the parameters from the previous paragraph and the fresh and dry mass of roots and leaves (Figures 3 and 4) found in this study may be related to the type of substances, functional group, chemical properties, and concentration in the medium in which the extracts of *J. curcas* leaves are acting (Goldfarb et al., 2009). Still, Reigosa et al. (2006) state that, the allelochemicals have the power to interfere with cell division, organic synthesis, hormonal interactions, nutrient uptake, protein synthesis, lipid metabolism changes, stomatal conductance, assimilation of CO_2 and electron transport in photosynthesis, as well as the content of chlorophyll in the plant.

Regarding the allelopathic power of *J. curcas*, in the study of Abugre et al. (2011), among all species evaluated, this was the one that most inhibited the okra germination (*Abelmoschus esculentus* (L.) Moench), with its root extracts having inhibited 53.3% of the seed lot and leaf extract 68.3%, which showed a stronger effect

Table 1. Mean results and standard deviations of the parameters evaluated on the initial growth of cauliflower.

(%)	(SD)	(SL)	(RL)	(LL)	(LA)
0	0.850 ± 0.067	1.895 ± 0.169	4.965 ± 0.421	2.755 ± 0.440	1.000 ± 0.486
5	1.000 ± 0.095	1.813 ± 0.178	5.073 ± 0.433	3.488 ± 0.635	1.937 ± 0.308
10	0.935 ± 0.181	1.973 ± 0.129	4.480 ± 0.413	4.092± 0.549	1.708 ± 0.557
20	0.950 ± 0.071	2.063 ± 0.218	4.550 ± 1.024	3.530 ± 0.295	1.500 ± 0.570
40	1.108 ± 0.120	2.196 ± 0.263	5.520 ± 0.835	4.353 ± 0.614	1.875 ± 0.268
80	1.075 ± 0.112	3.098 ± 0.346	4.706 ± 0.429	3.973 ± 0.420	1.867 ± 0.417
40	35.3	15.9	11.1	59.7	87.5
80	26.5	67.0	-5.2	42.3	86.7

%: percentage of variation, SD: stem diameter, SL:stem length, RL: root length, LL: leaf length, LA: leaf area.

of the leaf extract compared to the root extract.Investigation on the photochemical of stem bark extracts of the plant *J. curcas* performed by Igbinosa et al. (2009) showed the presence of some secondary metabolites such as steroids, tannins, glycosides, alkaloids, and flavonoids. Begum et al. (2011) identified ƒβ-sitosterol-3-O-ƒβ-Dglucopyranoside (daucosterin), 7-keto-ƒβ-sitosterol, and ƒβ- sitosterol through analyses in leaf extracts. For this species, phenolic compounds are the main substances responsible for exercising allelopathic effect, whose high concentrations of leaf and root extracts were found to inhibit the growth of seedlings of beans (*Phaseolus vulgaris* L.), corn (*Z. mays* L.), tomato (*Solanum lycopersicum* L.) and okra (*A. esculentus* (L.) Moen*ch) (*Abugre and Sam, 2010).

On the other hand, the use of products extracted from plants can be a strong ally to other methods of insect control, maintaining the environmental balance, leaving no chemical residue, no toxic effects on animals and humans, and reducing the negative effects caused by the uncontrolled application of organosynthetic insecticides (Machado et al, 2007).

Thus, even checking that the species *J. curcas* exercised positive activity on most of these parameters in the laboratory, it is necessary to carry out field studies to prove these effects and also its intensity (Corsato et al., 2010), once in addition to the aforementioned effects, there is possibility of allelochemicals transforming themselves in the environment due to the action of microorganisms, as well as other factors present in the upper layer in the soil, in substances with completely different chemical properties, which can be beneficial or detrimental to neighboring plants (Ferreira and Borghetti, 2004).

Conclusion

The conditions under which this experiment was conducted, there was no statistical effect on the root growth of cauliflower; however, the diameter, stem length, leaf length, leaf area, fresh and dry mass of leaves, and roots were influenced positively by the high concentrations of crude aqueous extract. According to the fitted values, the maximum value for mass gain would be achieved with levels of substrate of 46.4% to leaves and 49.2 to the roots.

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