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Review

Maize streak virus: A review of pathogen occurrence, biology and management options for smallholder farmers

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Maize streak disease is a major threat to cereal crops amongst smallholder farmers in sub-Saharan Africa causing up to US$480 million losses annually. It is caused by *Maize streak virus* (MSV), a geminivirus that is indigenous to Africa. The virus is transmitted by at least 11 *Cicadulina* species, with *Cicadulina mbila* being the main vector. In addition to cereals, the virus also infects wild grasses. There are 11 known MSV strains, designated with the letters A to K, according to alphabetical order. MSV-A is the most severe and economically important strain that attacks maize. The other strains attack cereal crops other than maize. The control of MSV is most effective when cultural and chemical methods are integrated with plant breeding for resistance. While host plant resistance is the best method of MSV management, it is not usually easy to conventionally produce resistant cultivars. Genetic engineering has been successfully employed in producing MSV-resistant maize. However, opponents of genetic engineering have prevented the adoption of the technology by most African countries. This means that smallholder farmers have to continue growing susceptible cultivars or buy the slightly more expensive conventionally-bred cultivars.

Key words: *Cicadulina*, host range, integrated disease management, pathogen strains, symptoms.

INTRODUCTION

Maize streak virus (MSV) is one of at least eight viruses that cause significant agronomic losses in maize (*Zea mays* L.) worldwide (Redinbaugh et al., 2004). It is the causative agent for maize streak disease (MSD), a major maize disease in sub-Saharan Africa (Ininda et al., 2006; Magenya et al., 2008; Martin and Shepherd, 2009) where it manifests from sea level to 2000 m above sea level (Welz et al., 1998). MSV is indigenous to Africa, including the adjacent Indian Ocean Islands of Reunion, Mauritius and Madagascar (Willment et al., 2001; Fajemisin, 2003). The first record of MSD was by Claude Fuller in 1901 in South Africa's Natal province (now KwaZulu-Natal) (cited by Shepherd et al., 2010). Serious MSV epidemics have been reported in at least 20 African countries including Angola, Benin, Burkina Faso, Cameroon, Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Zambia and Zimbabwe (Wambugu and Wafula, 2000; Lagat et al., 2008; Magenya et al., 2008). MSD is

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the most important maize disease in Zimbabwe where it occurs in every natural farming region. The disease is more prevalent on the Highveld (1219 to 1675 m above sea level) and Middleveld (600 to 1200 m above sea level) where farmers grow maize and winter cereals in rotation.

Yield losses by MSV are estimated from trace to 100% depending on cultivar and time of infection (Alegbejo et al., 2002). This translates to between US$120 million and US$480 million per year (Martin and Shepherd, 2009). In Kenya, food security in the smallholder sector is greatly compromised as annually, up to one million metric tonnes of maize grain is lost to MSD (Ininda et al., 2006). The smallholder farmers are particularly vulnerable to MSV because many of them grow susceptible traditionally open-pollinated varieties because they cannot afford to buy MSV-resistant hybrids. Often, such varieties are killed if infection occurs within three weeks after emergence.

This paper reviews the biology and control of MSD. It characterizes the MSV pathogen, its vectors, host range and symptoms. In view of the significance of the smallholder agricultural sector to sub-Saharan Africa, the paper evaluates the different control options available to farmers for dealing with the disease. The strengths and limitations of each control option are highlighted.

MAIZE STREAK VIRUS (MSV) BIOLOGY

Pathogen characterization

MSV is one of at least nine currently known grass infecting “African streak virus” species, a group that includes *Panicum streak virus*, *Sugarcane streak virus*, *Sugarcane streak Egypt virus*, *Eragrostis streak virus*, *Sugarcane streak Reunion virus, Urochloa virus* (cited by Martin and Shepherd, 2009), *Eragrostis minor streak virus* (Martin et al., 2011) and *Axonopus compressus streak virus* (Oluwafemi et al., 2014). It belongs to the genus *Mastrevirus* in the family *Geminiviridae*. It is a DNA virus with monopartite genome consisting of circular single-stranded DNA encapsidated in a characteristic geminate morphology (Muhire et al., 2013). Virion particles have a quasi-icosahedral shape, 27 x 38 nm (Shepherd et al., 2010). MSV is the reference species of the genus *Mastrevirus* whose other important species include *Bean yellow dwarf virus* (BeYDV), *Chloris striate mosaic virus* (CSMV), *Digitaria streak virus* (DSV), *Tobacco yellow dwarf virus* (TYDV) and *Wheat dwarf virus* (WDV).

MSV is the only species known to cause MSD. There are 11 known strains of MSV namely MSV-A to MSV-K (Shepherd et al., 2010; Monjane et al., 2011). The MSV-A strain causes the most severe and economically relevant form of MSD. It has five strain variants namely: MSV-A_1, MSV-A_2, MSV-A_3, MSV-A_4 and MSV-A_6. These variants have different geographical ranges. MSV-A_1 is the most widely distributed, occurring in every part of sub-Saharan Africa. Zimbabwe has MSV-A_1 and MSV-A_4 variants while South Africa has MSV-A_1, and MSV-A_4, in addition to MSV-B, MSV-C, MSV-D and MSV-E (Martin et al., 2001; Varsani et al., 2008). The MSV-A_1 and MSV-A_2 variants produce the severest symptoms; MSV-A_3 and MSV-A_6 produce intermediate symptoms and MSV-A_4 produces the mildest symptoms (Magenya et al., 2008). The MSV-B to MSV-K strains infect crops other than maize (Willment et al., 2002).

Vectors

MSV is obligately transmitted by several species of leafhoppers of the genus *Cicadulina* (Cicadelidae: Homoptera) in a persistent manner (Bosque-Perez et al., 2000). The most important vector is *Cicadulina mbila* (Naude) which has a wider geographical range and greater capacity to transmit the virus than any of other leafhopper species. The other confirmed vectors are *Cicadulina storeyi* China, *Cicadulina arachidis* China, *Cicadulina bipunctata* (Melichar), *Cicadulina latens* (Fennah), *Cicadulina parazeae* Ghauri, *Cicadulina similis* China, *Cicadulina triangular* Ruppel, *Cicadulina ghauri* China (Lett et al., 2002; Fajinmi et al., 2012), *Cicadulina chinai* Ghauri (www.grainsa.co.za) and *Cicadulina dabrowskii* Webb (Oluwafemi et al., 2007). The *Cicadulina* species are generally considered as grassland species. They are present in wild and pasture throughout the year, but can migrate in large numbers to maize (Page et al., 1999). Female leafhoppers are two to three times more capable of transmitting the virus than males (Oluwafemi et al., 2007). Fertilized leafhoppers prefer wild grassed for oviposition. MSV is neither seed borne nor mechanically transmissible.

Host range

Besides maize, MSV infects other crops such as rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), finger millet (*Eleusine coracana* L.), pearl millet (*Pennisetum typhoides* L.), sorghum (*Sorghum bicolor* L.) and sugarcane (*Saccharum officinarum* L.) (Damsgeegt, 1983; Willment et al., 2001; van Antwerpen et al., 2011). Maize is a staple food crops in Africa, while sorghum, finger and pearl millet are major food crops especially in marginal areas of sub-Saharan Africa. Sugarcane is an important cash crop in a number of countries including Mauritius, South Africa and Zimbabwe. Wheat, barley and oats are grown as winter cereals in southern Africa thereby ensuring that the virus has favourable hosts throughout the year. MSV also attacks a wide range of grasses in the genera *Axonopus,*

Figure 1. Maize plants infected by *Maize streak virus.* Photograph by Charles Karavina.

Brachiaria, Coix, Eleusine, Paspalum, Imperata, Rottboellia, Eragrostis and *Setaria* (Willment et al., 2001; Lett et al., 2002; Fajemisin, 2003). Most of these grasses grow naturally in vleis and irrigation schemes.

SYMPTOMS

MSV symptoms have been described in detail by various researchers (Ward et al., 1999; Shepherd et al., 2010). Symptoms vary according to the MSV isolate. The symptoms are characterized by broken to almost continuous chlorotic stripes centred on the tertiary leaf veins (Pinner et al., 1988). They first manifest as minute pale circular spots on the lowest exposed part of the leaf. Only new leaves develop the symptoms of virus infection while leaves below the point of infection remain healthy (Hill and Waller, 1998). The spots develop into discontinuous pale yellow streaks, up to several millimetres in length, along the blades, parallel to the veins or broken chlorotic streaks on secondary or tertiary veins with primary veins being less affected than secondary and tertiary veins. The longitudinal chlorotic streaking causes a concomitant reduction in photosynthetic area, growth and yield of the plant. The streaks often fuse laterally to give narrow broken chlorotic stripes which may extend over the entire length of fully affected leaves. In highly susceptible genotypes, chlorotic streaks tend to coalesce to form an almost uniform chlorosis. The chlorosis is caused by failure of chloroplasts to develop in the tissue surrounding the vascular bundles and this results in reduced photosynthesis and increased respiration leading to reduction in leaf length and plant height. Thus maize plants infected within the first three weeks after emergence become severely stunted producing considerable abnormal cobs or giving no yield at all. If infection occurs more than eight weeks after plant emergence, the virus does not normally cause significant economic loss (Page et al., 1999). Figure 1 shows typical MSV symptoms on maize plants.

MANAGEMENT OF MAIZE STREAK DISEASE

Numerous options have been recommended for the management of MSD. Current management strategies rely on the employment of cultural, chemical and host plant resistance measures. The availability, feasibility and cost-effectiveness of each method differ with production regions and settings, that is, commercial or subsistence (Pratt et al., 2003). Farmers are encouraged to combine at least two different tactics in dealing with the disease, a concept called Integrated Disease Management (IDM).

Cultural control

Various cultural practices are currently employed in the management of MSV. These include crop rotation, field hygiene, early planting and cultivar choice. Rotation should be practiced with broadleaved crops because MSV does not infect broadleaved plants at all (Damsteegt, 1983; Shepherd et al., 2010). Smallholder farmers commonly grow broadleaved crops like groundnuts (*Arachis hypogeae*), beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*), cotton (*Gossypium hirsutum*) and pumpkin (*Cucurbita pepo*). Recently, more and more Zimbabwean smallholder farmers have diversified their production into tobacco (*Nicotiana tabacum*) and soybean (*Glycine max*) which are non-hosts of MSV. A major impediment to the implementation of crop rotation amongst smallholder farmers is the land sizes which average 0.5 to 1.5 ha per farmer. With such small pieces of land, farmers tend to prioritize the growing of staple crops over MSV management. A better alternative to crop rotation is intercropping. Farmers can grow cereal food crops intercropped with legumes and cucurbits. However, intercropping reduces the yield of the main cereal crop (Page et al., 1999).

Common field hygienic measures for MSD management are roguing and the destruction of weeds, volunteer and ratoon crops. Infected crops should be rogued and buried as soon as they are identified during the growing season. Grassy weeds and cereal volunteer crops should also be destroyed. Normally, smallholder farmers are tempted to maintain ratoon and volunteer crops because they do not have adequate financial resources to purchase certified seed every season. If these are infected, then they act as sources of inoculum for MSV.

In the commercial farming sector, farmers maintain a buffer zone of five to ten meters around the field to reduce the movement of leafhoppers and subsequent virus spread. This zone has to be sprayed regularly with insecticides; or the grass in the buffer has to be kept short by regular mowing. A similar zone can be maintained between early and late planted crops. The smallholder farmer does not have the luxury of maintaining such a buffer zone because of limited land size. Neither can the farmer afford to purchase insecticides to spray onto the buffer. However, should they opt for such a buffer zone, they can use it to grow MSV non-host crops like groundnuts, beans and cucurbits in order to maximize land use.

Early planting is also recommended for MSV management (Magenya et al., 2008; Shepherd et al., 2010). An early planted crop grows past the susceptible stages before leafhopper population has built up sufficient levels to spread the virus. Also, the crop also grows vigorously as it is able to access high heat units in the early months of the summer season. However, planting dates are largely determined by the onset of the rainy season, especially in the smallholder sector where there are no irrigation facilities. So, with climate change, it is difficult to recommend this option because smallholder farmers are normally forced to plant with the first effective rains. In Zimbabwe, for example, the rainy season is now effectively starting in early December as opposed to early or mid-November. By this time, some non-effective early rains would have led to the emergence of wild grasses, ratoon and volunteer crops. If these are infected with the virus, then they provide MSV inoculum for the cereal crop to be planted at the onset of the rainy season.

In areas where MSD pressure is high, it is better to plant short season varieties as they are exposed to the disease over a shorter time than long season varieties. Farmers should avoid planting maize downwind of older cereal crops. Leafhoppers will be easily carried downwind and infect the new crop.

Chemical control

The application of insecticides is aimed at controlling the vectors. Insecticides like aldicarb, carbofuran, carbosulfan, dimethoate, endosulphan and imidacloprid are available for use against leafhoppers. The most commonly used insecticides are carbofuran and imidacloprid. Carbofuran can be applied as seed dressing, in planting farrows or as conventional sprays (Magenya et al., 2008). It also controls other maize pests like maize stalk borer (*Busseola fusca* (Fuller)), termites (*Microtermes spp* and *Macrotermes spp*), white grubs (*Adoretus spp*), and wireworms (*Agriotes obscurus* L.). With a residual activity of up to seven weeks, carbofuran protect the maize crop past the susceptible stages.

Imidacloprid is mainly applied as seed dressing when controlling leafhoppers. It is another broad spectrum insecticide that has many formulations. It is marketed as Gaucho, Confidor, Admire or Conguard. In Zimbabwe, Gaucho is used for seed treatment while Confidor is formulated for foliar sprays. Two organophosphorus insecticides dimethoate and endosulphan are commonly applied as foliar sprays to control leafhoppers in the field and buffer zone. Sometimes, farmers spray herbicides like glyphosate and paraquat in the buffer zone to kill the grass thereby creating bare ground that repels the leafhoppers.

There are several challenges associated with chemical control of MSD. Pesticides like aldicarb, paraquat and carbofuran are extremely poisonous to both humans and the environment. Aldicarb and paraquat are part of the “Dirty Dozen”, a list of chemicals that have been banned in the developed world (www.legacy.library.ucsf.edu). However, they are still in use most developing countries where technically and economically feasible alternatives with acceptable health and environmental effects are not available.

Besides their effects on humans and the environment, foliar pesticide sprays quickly break down during the growing season. So, they need to be repeatedly applied throughout the growing season for effective leafhopper control. Most smallholder farmers are financially constrained to buy pesticides. Even when they have the pesticides, they usually do not have adequate protective clothing and technical knowhow on correct pesticide application techniques. As a result, there have been many deaths due to pesticide poisoning. The World Health Organization estimates that one million people are poisoned annually with 20,000 cases resulting in deaths (Matthews et al., 2003). In most cases, smallholder farmers are usually far away from medical services in case there is accidental poisoning. Sometimes, the medical services are poorly equipped to handle cases of pesticide poisoning.

Host plant resistance

By far the most effective, economically viable and environmentally friendly method of MSV management is the growing of resistant cultivars (Lagat et al., 2008). Resistant cultivars are produced through either conventional breeding or genetic engineering.

Conventional breeding

Breeding for MSV resistance is done by the private sector, international research centers and national programmes (Pratt et al., 2003). The International Maize and Wheat Improvement Centre (CIMMYT) and International Institute of Tropical Agriculture (IITA) are the major international research centers involved in breeding for MSV resistance in Africa. They have identified germplasm that has high tolerance to MSV, with the *msv-1* gene responsible for conferring the resistance (Kyetere et al., 1995). Private companies and government programmes involved in breeding MSV-resistant hybrids get germplasm from these international research centers. CIMMYT has regional offices in Harare (Zimbabwe) and Nairobi (Kenya) servicing southern and East Africa, respectively. IITA has its offices in Nigeria, and it services West Africa.

In view of the severity of MSV in sub-Saharan Africa, all maize breeding programmes incorporate resistance to MSV. To date, several MSV-tolerant cultivars have been released throughout the sub-Saharan region. In Zimbabwe, for example, SeedCo (a private company) has released the cultivars SC403, SC411, SC621, SC713 and SC719 which are being marketed in a number of countries in the region (SeedCo Manual, 2010-2011). The yield and quality of the tolerant cultivars are comparable to those of susceptible cultivars. However, they tend to be slightly more expensive than susceptible ones. Therefore, farmers should be prepared to pay slightly more for tolerant cultivars. In most African countries though, the price of maize seed is subsidized by government to make it affordable even to the poorest farmers.

There are several challenges in producing conventionally bred maize genotypes with a high degree of resistance. The MSV resistance reported so far relies heavily on the *msv-1* gene. If MSV were to evolve and yield strains capable of overcoming the *msv-1* gene, all currently resistant germplasm would be rendered ineffective (Redinbaugh et al., 2004; Olaoye, 2009). Also, resistance may be associated with undesirable traits like low yield and poor taste. Thus, farmers have to make choices between MSV resistance versus desirable agronomic and nutritional traits. Another major challenge is that resistance genes are scattered amongst different chromosomes. This makes it difficult to transfer them to agronomically favourable genotypes because of linkage drag (Martin and Shepherd, 2009). In most sub-Saharan countries, there are no effective seed multiplication and distribution systems for the resistant cultivars (Thresh, 2003).

Genetic engineering

Genetic engineering is the direct manipulation of an organism's genome using biotechnology. In this instance, either natural resistance genes or resistance genes from other sources are inserted into a crop host to produce a genetically modified plant. The concept of pathogen derived resistance (PDR) proposed by Sanford and Johnston (1985) has been effectively used to produce MSV-resistant maize in South Africa. Shepherd et al. (2007) used dominant negative mutants of the MSV replication-associated protein gene (rep) to develop resistance in maize. This was the first time maize with transgenic MSV-resistance was developed anywhere in the world (Sinha, 2007). There are several drawbacks with genetic engineering for producing MSV-resistant maize; the main one being the unfavourable perception on genetically engineered foods the world over (Arthur, 2011; Adenle et al., 2014). In Africa, only Burkina Faso, Egypt, South Africa and Sudan permit genetically modified (GM) crop farming (James, 2013). Zimbabwe, like most other sub-Saharan countries, banned GM cropping despite regularly importing agricultural products like maize mealie-meal and cooking oil from South Africa, a country that has embraced the GM technology. The imports are necessitated by food deficits caused by frequent droughts and a decline in domestic agricultural output. While there have been no major reported MSV epidemics in the last twenty years, it does not mean that the MSD problem has been overcome. Disease epidemics are likely to occur in future, especially if the *msv-1* gene is overcome. As such, farmers should be allowed to choose between susceptible conventionally-bred maize and transgenic resistant maize varieties. While not advocating for conventional breeding to be scrapped out, it is my view that transgenic maize should be given a chance to be on the market. Most of the fears about GM crops are based on perceptions rather than true scientific facts.

CONCLUSION

MSD remains an important disease in sub-Saharan Africa. In spite of no major, widely reported epidemics having occurred in the last two decades, the disease is there to stay in the region. With climate change and more land being utilized for cereal cropping, it is most likely that the next major epidemic is just around the corner. The smallholder farmer who is mainly dependent on cereal crops for food is more exposed to such epidemics when they occur. In the meantime, an integrated approach to MSV management is the best way of dealing with the disease.

Conflict of Interest

The authors have not declared any conflict of interest.

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Pre-breeding of elephant grass for energy purposes and biomass analysis in Campos dos Goytacazes- RJ, Brazil

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The objective of this study was to evaluate the morpho-agronomic traits of eighty five accessions of elephant grass (*Pennisetum purpureum* Schum.) from the germplasm bank located at the Experimental Field of the State Center for Research on Bioenergy and Waste Recovery (*Centro Estadual de Pesquisa em Agroenergia e Aproveitamento de Resíduos*). The experimental design was a randomized block with two replicates, in an annual-harvest system. The evaluated traits were: percentage of dry matter (%DM), dry matter yield (DMY), number of tillers per linear meter (NT), plant height (HGT), stem diameter (SD), leaf blade width (LW) and leaf blade length (LL). Each cut and variable underwent variance analysis and the Scott-Knott test (P<0.05). Tocher's optimization method, Mahalanobis distance and canonic variables were used for the multi-traits, and importance of the characters in the canonic variables. The following elite genotypes were identified by the Scott-Knott test at 5%: Rico 534-B, Taiwan A-144, Napier S.E.A., Mole de Volta Grande, Teresópolis, Taiwan A-46, Duro de Volta Grande, Turrialba, Taiwan A-146, Cameroon Piracicaba, Taiwan A-121, P241 Piracicaba, Elefante Cachoeira Itapemirim, Guaco/I.Z.2, Cameroon, IJ 7126 cv EMPASC 310, IJ 7139, Australiano, 10 AD IRI, and Pasto Panamá. By the analysis of the canonic variables, the first two accumulated 64.6457% of variance. Regarding the relative importance of the evaluated traits, the leaf blade length at cut two was the most important. By Tocher's optimization method, the eighty five accessions were divided into twenty five groups, indicating a high variability in the germplasm bank.

Key words: *Pennisetum purpureum*, Tocher's optimization, Mahalanobis distance, elephant grass.

INTRODUCTION

The search for alternatives to reduce the use of fossil fuels for energy production has increased worldwide in the past years due to the elevated international price of oil and its derivatives, and especially due to the concerns with the environmental and climatic changes (Morais et al., 2009). A prominent alternative today is the use of

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plant biomass as a source of energy insofar as its combustion only recycles the CO_2 that has been removed from the atmosphere by photosynthesis (Quesada et al., 2003).

Pennisetum purpureum Schum., popularly known as elephant grass, is a native perennial *Poaceae* from tropical Africa, common to the fertile valleys between the latitudes 10° N and 10° S, having an annual precipitation of over 1,000 m. It was discovered by Colonel Napier in 1905 and later spread throughout Africa. In 1920 it was introduced in Brazil, from Cuba (Carvalho et al., 1995; Deresz, 1999; Rodrigues et al., 2001; Pereira et al., 2008; Lima et al., 2010). This forage plant was well-disseminated all over the country in view of its good adaptation to the tropical environment, growing well from the sea level to altitudes of 2,200 m, between temperatures of 18 and 30°C and precipitation of 800 to 4,000 mm (Pereira et al., 2008; Lima et al., 2010).

The elephant grass is among the species with high photosynthetic efficiency, which results in a great capacity to accumulate dry matter (Queiroz Filho et al., 2000; Quesada et al., 2003; Boddey et al., 2004). Thus, in the recent years researchers have demonstrated a great interest in producing energy from plant biomass (Quesada et al., 2004).

This objective would require further studies on the identification of elephant-grass genotypes, especially so as to reveal accessions with ideal traits for biomass production aiming at energy purposes (Cavalcante and Lira, 2010; Zanetti, 2010), which can be utilized in breeding programs.

Therefore, it is extremely important to insert and maintain this species in a germplasm bank to store and provide accessions, preserve the genetic variability for the future, provide information on the accessions and identify traits of interest for breeding programs (Nass and Paterniani, 2000). However, it is known that there is a low use of the plant genetic resources in germplasm banks due to the lack of documentation, description and proper evaluation of the collections (Nass and Paterniani, 2000). The genetic divergence can be based on morphological traits which will guide the targeted crosses aiming to maximize the hybrid vigor (Schneider, 2013). Based on the foregoing, the objective of this study is to estimate the genetic diversity among 85 accessions of elephant grass through morphological traits and considering the annual productivity of the accessions so as to identify the elite genotypes, providing bases for the pre-breeding activities to guide planned crosses.

MATERIALS AND METHODS

Installation and development of the experiment

The experiment was conducted at State Center for Research on Bioenergy and Waste Recovery (*Centro Estadual de Pesquisas em Agroenergia e Aproveitamento de Resíduos*), located in the city of Campos dos Goytacazes (coordinates: 21° 19' 23'' S latitude and 41° 19' 40'' W longitude; elevation of 20 to 30 m). The climate in this region, according to the Köppen (1948) classification, is an Aw (hot and humid tropical); dry in the winter, rainy in the summer, and with an annual precipitation of approximately 1.152 mm. The soil is classified as a Yellow Oxisol, which has the following characteristics: pH, 5.5; phosphorus, 18 mg dm^{-3}; potassium, 83 mg dm^{-3}; Ca, 4.6 cmolc dm^{-3}; Mg, 3.0 cmolc dm^{-3}; Al, 0. 1 cmolc dm^{-3}; H + Al, 4.5 cmolc dm^{-3}; and C 1.6%.

The genetic material consists of 85 genotypes of elephant grass (Table 1) from the Active Germplasm Bank of Elephant Grass (BAG - CE) of Embrapa Dairy Cattle, located in Coronel Pacheco/MG. The germplasm bank was planted in February, by distributing whole stems into the furrows, two per furrow, positioned with their base in contact with the apex of the next plant. After this distribution in the furrows, they were cut into pieces containing two or three buds. An experimental design of randomized blocks with two replicates was adopted, and the plot was formed by a 5.5-m row with 2-m spacing, totaling 11 m^2. The floor area utilized was a sample collected from the center of the plot.

In fertilization at planting, each row received 60 g of single superphosphate, and 50 days after planting topdressing was performed with 70 g of urea and 40 g potassium chloride (KCl) per row and 24 kg K_2O (potassium oxide) per hectare. After the establishment phase, on December 15, 2011, plot-leveling and replanting were performed to reduce flaws in the rows. The first cut was performed after one year of growth, on 11/27/2012; the second cut was made on 11/05/2013, after another year of growth.

Evaluated traits

Morpho-agronomic traits were evaluated in all genotypes after each year of continuous growth, with whole-plant samples.

Morpho-agronomic traits

(a) Dry matter yield (DMY): The biomass of each plot was weighed, and then sub-samples were collected, chopped, and conditioned in paper bags to be dried in a forced-air oven at 65°C. After 72 h the samples were once again weighed to obtain the result of air-dried samples (ADS).

(b) Percentage of dry matter (%DM): Two grams of the ADS were ground in a Wiley mill (1 mm sieve) and then dried in an oven for 16 h at 105°C to obtain the oven-dried sample (ODS). The percentage of DM was estimated from the result of the air- (ADS) and oven-dried (ODS) samples.

(c) Number of tillers (NT): The number of tillers was counted in 1.5 linear meters of one of the rows of the plot, and subsequently converted to number of tillers per linear meter.

(d) Plant height, in m (HGT): The height was measured with a tape measure, taking the average of three measurements in each plot.

(e) Average diameter of the stem at the base of the plant, in cm (SD): The measurements were made approximately 10 cm above the soil, obtaining the average of three measurements using a digital caliper.

(f) Leaf blade width and length, in cm (LW and LL, respectively): Measured using a centimeter ruler, taking the average of three measurements.

Statistical analyses

The obtained data, for each trait, were subjected to variance analysis using the GENES (Cruz et al., 2013) computer software, according to the following statistical model:

$$Yij = M + Gi + Bj + eij,$$

Table 1. Genotypes of elephant grass from the Active Germplasm Bank of Elephant grass (BAG-CE), Campos dos Goytacazes, RJ, 2012/2013.

S/No	Genotype	Origin	S/No	Genotype	Origin
1	Elefante da Colômbia	Colombia	44	Capim Cana D'África	Brazil
2	Mercker	Brazil	45	Gramafante	Brazil
3	Três Rios	Brazil	46	Roxo	Brazil
4	Napier Volta Grande	Brazil	47	Guaco/I.Z.2	Brazil
5	Mercker Santa Rita	Brazil	48	Cuba-115	Cuba
6	Pusa Napier N 2	Índia	49	Cuba-116	Cuba
7	Gigante de Pinda	Brazil	50	Cuba-169	Cuba
8	Napier N 2	Brazil	51	King Grass	Cuba
9	Mercker S.E.A	Brazil	52	Roxo Botucatu	Brazil
10	Taiwan A-148	Brazil	53	Mineirão IPEACO	Brazil
11	Porto Rico 534-B	Brazil	54	Vruckwona Africano	Brazil
12	Taiwan A-25	Brazil	55	Cameroon	Brazil
13	Albano	Colombia	56	CPAC	Brazil
14	Hib, Gigante Colômbia	Colombia	57	Guacu	Brazil
15	Pusa Gigante Napier	Índia	58	Napierzinho	Brazil
16	Elefante Híbrido 534-A	Brazil	59	IJ 7125 cv EMPASC 308	Brazil
17	Costa Rica	Costa Rica	60	IJ 7126 cv EMPASC 310	Brazil
18	Cubano Pinda	Brazil	61	IJ 7127 cv EMPASC 309	Brazil
19	Mercker Pinda	Brazil	62	IJ 7136 cv EMPASC 307	Brazil
20	Mercker Pinda México	Brazil	63	IJ 7139	Brazil
21	Mercker 86 México	Colombia	64	IJ 7141 cv EMPASC 306	Brazil
22	Taiwan A-144	Brazil	65	Goiano	Brazil
23	Napier S.E.A,	Brazil	66	CAC-262	Brazil
24	Taiwan A-143	Brazil	67	Ibitinema	Brazil
25	Pusa Napier N 1	Índia	68	903-77 ou Australiano	Brazil
26	Elefante de Pinda	Colombia	69	13 AD	Brazil
27	Mineiro	Brazil	70	10 AD IRI	Brazil
28	Mole de Volta Grande	Brazil	71	07 AD IRI	Brazil
29	Porto Rico	Brazil	72	Pasto Panamá	Brazil
30	Napier	Brazil	73	BAG - 92	Brazil
31	Mercker Comum	Brazil	74	09 AD IRI	Brazil
32	Teresopólis	Brazil	75	11 AD IRI	Brazil
33	Taiwan A-46	Brazil	76	05 AD IRI	Brazil
34	Duro de Volta Grande	Brazil	77	06 AD IRI	Brazil
35	Mercker Comum Pinda	Brazil	78	01 AD iIRI	Brazil
36	Turrialba	Brazil	79	04 AD IRI	Brazil
37	Taiwan A-146	Brazil	80	13 AD IRI	Brazil
38	Cameroon - Piracicaba	Brazil	81	03 AD IRI	Brazil
39	Taiwan A-121	Brazil	82	02 AD IRI	Brazil
40	Vrukwona	Brazil	83	08 AD IRI	Brazil
41	P241 Piracicaba	Brazil	84	União	Brazil
42	IAC-Campinas	Brazil	85	Pesagro Bord	Brazil
43	Elefante Cachoeira Itapemirim	Brazil			

where:

Y_{ij}: observation of the i-th genotype in the j-th block;
m: overall constant associated with this random variable;
G_i: effect of the i-th genotype;
B_j: effect of the j-th block;
e_{ij}: experimental error associated with observation Y.

Subsequently, the mean values of the genotypes were clustered for each variable within each evaluation, utilizing the Scott and Knott

Table 2. Summary of the variance analysis in randomized blocks for percentage of dry matter (%DM) and dry matter yield (DMY) at cuts 1 and 2, and total dry matter yield in t. ha^{-1} evaluated in 85 genotypes of elephant grass.

Source of variability	DF	Mean squared				
		Cut 1		Cut 2		Total
		%DM	DMY	%DM	DMY	DMY
Blocks	1	15.72	60.57	1.24	152.91	20.99
Treatments	84	63.03*	336.90**	21.38*	388.20*	1141.69**
Residue	84	43.27	167.36	14.38	259.48	593.98
Mean		35.67	35.01	36.73	41.32	76.33
CV (%)		18.43	36.95	10.33	38.98	31.93

** and * - significant at 1 and 5% probability, respectively, by the F test.

(1974) clustering method. And for multivariate analyses, we used: the canonic variables, eigenvalues and eigenvectors, Mahalanobis distance (D_2) and Tocher's clustering method. All analyzes were obtained with the aid of the GENES computer software (Cruz, 2013).

Clustering was performed via Tocher's optimization method via Mahalanobis distance (D_2), which adopts the criterion that the average of dissimilarity measures, within each group, should be smaller than the distances between any groups. By the dissimilarity matrix, we identify the pair of most similar accessions; these accessions will form the initial group, in which the possibility of including new accessions will be evaluated (Cruz and Regazzi, 2001).

RESULTS AND DISCUSSION

Variance analysis and mean clustering by the Scott-Knott test for percentage of dry matter and dry matter yield

The results demonstrate that there were significant differences by the F test at 1% probability for dry matter yield (DMY), in t.ha^{-1} at cut 1 (year 2012) and in the total; however, at cut 2 it was significant at 5% probability (year 2013). Still, the percentage of dry matter (%DM) from both cuts showed significance at 5% probability by the F test (Table 2).In a study conducted by Italiano et al. (2006) with ten genotypes of *P. purpureum*, it was found that at 60 days of age DMY was also significant at 1% probability.

The significant differences observed between the means of accessions of *P. purpureum*, regarding %DM and DMY, indicate that there is genetic variability in the active collection of germplasm bank, making it possible to select the best genotypes (Araujo et al., 2008). The experimental coefficients of variation (CV) for the DMY of cut 1, cut 2 and the total were similar; however, the lowest CV for DMY was found in the total (31.93%), whereas the highest value was observed in cut 2 (38.98%). On the other hand, the CV for %DM was lower than that of DMY, with 18.43% in cut 1 and 10.33% in cut 2 (Table 2).

The coefficients of variation indicate the precision of the experiment, and in agricultural field trials they can be considered low when below 10%, medium from 10 to 20%, high from 20 to 30%, and very high when above 30% (Fonseca and Martins, 1996); thus, the coefficient of variation for the two %DM values were medium, whereas the three values for DMY were considered very high, according to this classification. However, it should be emphasized that although the CV was very high for the DMY variable, it can be justified by the fact that three distinct variables are related to estimate this character: fresh-matter weight of the plot, ADS (sample dried at 65°C) and ODS (sample dried at 105°C). Other studies conducted with elephant grass have presented CV considered high or very high for DMY (Daher et al., 2004; Oliveira et al., 2012; Oliveira, 2013). However, %DM has shown a lower CV, ranging from low to medium (Oliveira et al., 2012; Oliveira, 2013), just as in the present study.

According to the data from the variance analysis (Table 2), the average dry matter yield in 2012 (35.01 t.ha^{-1}) was similar to that of 2013 (41.32 t.ha^{-1}). This was likely because there was no large variation between the precipitations of 2012 (781.1 mm) and 2013 (907.8 mm) (Table 3). Several studies have already demonstrated that the amount of available water interferes with the productivity of the plant, whose development improves as the availability of water is increased (Daher et al., 2000; Ribeiro et al., 2009; Vitor et al., 2009).

Although in the variance analysis the %DM showed a significant difference ($P<0.05$) at both cuts (Table 2), there were no significant differences among the genotypes by the Scott-Knott test at 5% for both cuts 1 and 2, and thus no groups were formed (Table 4). Despite the lack of significant difference, in cut 1 the %DM varied from 50.94 to 20.48% in the genotypes Napierzinho and Napier Volta Grande, respectively.

However, in cut 2 the highest %DM, 44.99% was found in genotype Australiano, and the lowest, 29.02%, in King Grass. Corroborating this result, in an evaluation with elephant grass at 51 days of growth, it was found that the %DM had a significant difference in the variance

Table 3. Monthly precipitation (mm) during the experiment.

2012		2013	
Month	**Precipitation (mm)**	**Month**	**Precipitation (mm)**
January	216.5	January	125.7
February	11.7	February	44.3
March	73.6	March	230.2
April	14.2	April	103.2
May	147.2	May	41.6
June	74.0	June	8.7
July	5.9	July	67.1
August	59.8	August	57.0
September	21.6	September	45.2
October	12.5	October	26.4
November	133.7	November	158.4
December	10.4	December	-
Total	781.1	Total	907.8

Source: Evapotranspiration Monitoring Station of the Centro Estadual de Pesquisa em Agroenergia e Aproveitamento de resíduos, Pesagro - Rio,Campos dos Goytacazes-RJ.

analysis ($P<0.05\%$), but this difference was not detected in the Scott-Knott test at 5% (Oliveira et al., 2012) A similar result was presented with 73 genotypes of *P. purpureum* at six months of age (Oliveira, 2013). Though in some studies no groups were formed by the Scott-Knott test at 5% (Oliveira et al., 2012; Oliveira, 2013), the analysis of the dry matter of each genotype (%DM) is very important, given that 90% of the plant cells may consist of water (Oliveira, 2013). For the variable DMY, at cut 1, the average production varied between 69.58 and 13.96 $t.ha^{-1}$, for genotypes Taiwan A-121 and Napier Volta Grande, respectively. The Scott-Knott clustering at 5% probability generated two groups, in which in 30 genotypes can be found in group "a", and the other 55 in group "b". In cut 2, the average DMY was between 78.01 $t.ha^{-1}$, for genotype Australiano, and 11.48 $t.ha^{-1}$, in genotype 07 AD IRI. Two groups were formed, with thirty three of them being in group "a", of 85 genotypes analyzed (Table 4). For total dry matter yield, just as in cut 2, the highest value was obtained by genotype Australiano, reaching 133.78 $t.ha^{-1}$, and genotype BAG-92 revealed the lowest mean, 32.11 $t.ha^{-1}$. In the Total, the groups formed were also two; however, group "a" had 39 genotypes in it. Thus, the genotypes considered elite were those that composed group "a" in cut 1, cut 2 and in the total, Porto Rico 534-B, Taiwan A-144, Napier S.E.A., Mole de Volta Grande, Teresópolis, Taiwan A-46, Duro de Volta Grande, Turrialba, Taiwan A-146, Cameroon-Piracicaba, Taiwan A-121, P241 Piracicaba, Elefante Cachoeira Itapemirim, Guaco/I.Z.2, Cameroon, IJ 7126 cv EMPASC 310, IJ 7139, Australiano, 10 AD IRI and Pasto Panamá. Based on that, these genotypes can be indicated in the future for possible crosses aiming to increase the genetic gain for this trait.

A similar study has been conducted (Oliveira, 2013) with seventy three of the elephant-grass genotypes described herein, and for the DMY variable the genotypes considered elite, and that corroborated this study were Taiwan A-46, Duro de Volta Grande, Guaco/I.Z.2 and Pasto Panamá; however, ten genotypes that were described as having good productivity were not considered elite genotypes in the current study. Just as in this study, genotype Cameroon has stood out for its productivity, and when compared with two other genotypes of elephant grass it was chosen as having the highest dry matter yield, for energy purposes (Quesada et al., 2004). This same result agrees with an analysis conducted with five other elephant-grass genotypes, which also concluded that Cameroon has one of the greatest dry-biomass yields, on a nine-month harvest system (Morais et al., 2009). Unlike these results, at ten months of growth, genotype Mercker 86-Mexico has gained prominence for producing 56.56 $t.ha^{-1}$ (Rossi, 2010).

To select the elite genotypes of elephant grass one must bear in mind that because it is a perennial culture, usually implanted to be utilized for some years, elephant-grass genotypes should be productive throughout the entire culture, and so it is more interesting to the produce that the genotypes have stable performance over the harvests (Souza Sobrinho et al., 2005).

Variance analysis of the morpho-agronomic traits

All morpho-agronomic traits evaluated showed significant differences by the F test ($P<0.01$), indicating that there is genetic variability in the active germplasm bank and also proving that the descriptors utilized among the genotypes demonstrated different degrees of discrimination. The

Table 4. Percentage of dry matter (%DM) and dry matter yield (DMY) at cuts 1 and 2, and total dry matter yield (cut 1 + cut 2) of the 85 genotypes of elephant grass.

Genotype	Cut 1		Cut 2		Total
	%DM (%)	DMY ($t.ha^{-1}$)	%DM (%)	DMY ($t.ha^{-1}$)	DMY ($t.ha^{-1}$)
Elefante da Colômbia	40.92 [a1]	28.67 [b]	36.66 [a]	27.48 [b]	56.15 [b]
Mercker	27.70 [a]	32.57 [b]	34.14 [a]	55.93 [a]	88.504 [a]
Três Rios	31.60 [a]	35.97 [b]	32.49 [a]	49.68 [a]	85.66 [a]
Napier Volta Grande	20.48 [a]	13.96 [b]	31.88 [a]	27.36 [b]	41.39 [b]
Mercker Santa Rita	33.00 [a]	30.09 [b]	39.04 [a]	32.41 [b]	62.51 [b]
Pusa Napier N 2	42.97 [a]	18.25 [b]	40.31 [a]	36.88 [b]	55.13 [b]
Gigante de Pinda	38.35 [a]	33.43 [b]	39.22 [a]	56.01 [a]	89.44 [a]
Napier N 2	44.20 [a]	17.23 [b]	40.38 [a]	22.17 [b]	39.40 [b]
Mercker S.E.A	49.95 [a]	26.87 [b]	39.93 [a]	31.70 [b]	58.56 [b]
Taiwan A-148	32.40 [a]	27.05 [b]	39.72 [a]	37.31 [b]	64.37 [b]
Porto Rico 534-B	38.99 [a]	41.02 [a]	36.96 [a]	60.50 [a]	101.52 [a]
Taiwan A-25	28.82 [a]	16.52 [b]	37.53 [a]	33.80 [b]	50.32 [b]
Albano	31.78 [a]	19.65 [b]	39.41 [a]	33.28 [b]	52.93 [b]
Hib, Gigante Colômbia	36.81 [a]	24.56 [b]	35.53 [a]	35.87 [b]	60.42 [b]
Elefante Híbrido 534-A	26.86 [a]	19.13 [b]	32.57 [a]	40.72 [b]	59.84 [b]
Costa Rica	32.66 [a]	28.02 [b]	33.85 [a]	36.48 [b]	64.50 [b]
Cubano Pinda	33.80 [a]	27.51 [b]	35.80 [a]	41.38 [b]	68.88 [b]
Mercker Pinda	42.86 [a]	27.28 [b]	37.47 [a]	41.57 [b]	68.85 [b]
Mercker Pinda México	34.56 [a]	20.78 [b]	39.28 [a]	40.15 [b]	60.93 [b]
Mercker 86 México	32.82 [a]	24.03 [b]	33.69 [a]	42.75 [b]	66.78 [b]
Taiwan A-144	39.28 [a]	47.54 [a]	37.16 [a]	58.75 [a]	106.29 [a]
Napier S.E.A.	41.32 [a]	48.87 [a]	40.46 [a]	54.87 [a]	103.74 [a]
Taiwan A-143	27.78 [a]	23.77 [b]	31.95 [a]	25.39 [b]	49.17 [b]
Pusa Napier N 1	31.85 [a]	29.04 [b]	32.34 [a]	58.38 [a]	87.42 [a]
Elefante de Pinda	31.06 [a]	24.86 [b]	35.76 [a]	35.35 [b]	60.21 [b]
Mineiro	31.99 [a]	41.14 [a]	31.29 [a]	34.74 [b]	75.88 [b]
Mole de Volta Grande	37.25 [a]	56.96 [a]	38.63 [a]	47.28 [a]	104.24 [a]
Porto Rico	36.11 [a]	36.93 [b]	42.20 [a]	59.68 [a]	96.61 [a]
Napier	33.98 [a]	36.32 [b]	41.40 [a]	60.83 [a]	97.16 [a]
Mercker Comum	37.69 [a]	23.34 [b]	37.53 [a]	31.44 [b]	54.78 [b]
Teresopólis	39.33 [a]	44.41 [a]	36.58 [a]	50.03 [a]	94.44 [a]
Taiwan A-46	34.93 [a]	63.96 [a]	38.12 [a]	58.56 [a]	122.51 [a]
Duro de Volta Grande	40.20 [a]	50.93 [a]	36.07 [a]	47.11 [a]	98.04 [a]
Mercker Comum Pinda	42.72 [a]	46.90 [a]	39.82 [a]	38.33 [b]	85.23 [a]
Turrialba	31.49 [a]	40.91 [a]	33.73 [a]	57.96 [a]	98.87 [a]
Taiwan A-146	31.37 [a]	39.13 [a]	31.22 [a]	49.33 [a]	88.46 [a]
Cameroon - Piracicaba	36.31 [a]	65.05 [a]	35.78 [a]	59.56 [a]	124.61 [a]
Taiwan A-121	41.17 [a]	69.58 [a]	38.96 [a]	57.53 [a]	127.11 [a]
Vrukwona	32.09 [a]	39.32 [a]	34.60 [a]	35.14 [b]	74.45 [b]
P241 Piracicaba	41.62 [a]	64.81 [a]	36.73 [a]	63.72 [a]	128.53 [a]
IAC-Campinas	32.65 [a]	43.73 [a]	36.39 [a]	38.00 [b]	81.73 [a]
Elefante Cachoeira Itapemirim	36.86 [a]	64.14 [a]	38.69 [a]	69.32 [a]	133.47 [a]
Capim Cana D'África	37.52 [a]	41.82 [a]	36.40 [a]	25.64 [b]	67.47 [b]
Gramafante	32.15 [a]	35.41 [b]	42.26 [a]	56.23 [a]	91.64 [a]
Roxo	34.24 [a]	31.26 [b]	34.14 [a]	26.00 [b]	57.26 [b]
Guaco/I.Z.2	31.51 [a]	48.19 [a]	38.30 [a]	62.04 [a]	110.24 [a]
Cuba-115	35.02 [a]	31.01 [b]	32.91 [a]	21.42 [b]	52.43 [b]
Cuba-116	35.39 [a]	53.45 [a]	37.07 [a]	41.66 [b]	95.11 [a]

Table 4. Contd.

Cuba-169	28.00 [a]	21.98 [b]	35.38 [a]	44.42 [a]	66.41 [b]
King Grass	36.16 [a]	53.94 [a]	29.02 [a]	29.70 [b]	83.64 [a]
Roxo Botucatu	44.11 [a]	28.86 [b]	29.96 [a]	27.08 [b]	55.94 [b]
Mineirão IPEACO	31.78 [a]	34.08 [b]	37.53 [a]	51.77 [a]	85.85 [a]
Vruckwona Africano	39.25 [a]	36.83 [b]	35.19 [a]	43.71 [b]	84.03 [a]
Cameroon	36.23 [a]	47.70 [a]	36.58 [a]	47.20 [a]	87.74 [a]
CPAC	37.61 [a]	36.65 [b]	33.55 [a]	40.04 [b]	64.11 [b]
Guacu	26.45 [a]	24.63 [b]	37.70 [a]	27.46 [b]	64.49 [b]
Napierzinho	50.945 [a]	32.4015 [b]	34.155 [a]	39.852 [b]	47.8945 [b]
IJ 7125 cv EMPASC 308	37.56 [a]	39.05 [a]	37.82 [a]	15.49 [b]	106.71 [a]
IJ 7126 cv EMPASC 310	38.95 [a]	50.92 [a]	37.77 [a]	67.66 [a]	88.56 [a]
IJ 7127 cv EMPASC 309	30.88 [a]	49.66 [a]	35.45 [a]	37.63 [b]	98.09 [a]
IJ 7136 cv EMPASC 307	31.83 [a]	20.06 [b]	35.43 [a]	48.43 [a]	71.87 [b]
IJ 7139	36.75 [a]	46.23 [a]	36.62 [a]	51.81 [a]	89.94 [a]
IJ 7141 cv EMPASC 306	28.26 [a]	22.27 [b]	35.59 [a]	30.55 [b]	52.82 [b]
Goiano	31.02 [a]	26.14 [b]	29.90 [a]	34.58 [b]	60.72 [b]
CAC-262	34.87 [a]	45.60 [a]	35.83 [a]	40.32 [b]	85.92 [a]
Ibitinema	34.11 [a]	37.71 [b]	41.87 [a]	49.36 [a]	87.06 [a]
903-77 ou Australiano	34.83 [a]	55.76 [a]	44.99 [a]	78.01 [a]	133.77 [a]
13 AD	40.54 [a]	32.03 [b]	36.75 [a]	28.75 [b]	60.78 [b]
10 AD IRI	36.99 [a]	40.35 [a]	39.59 [a]	52.07 [a]	92.42 [a]
07 AD IRI	35.99 [a]	20.97 [b]	38.31 [a]	11.48 [b]	32.45 [b]
Pasto Panamá	34.13 [a]	44.01 [a]	31.63 [a]	50.34 [a]	94.35 [a]
BAG - 92	37.87 [a]	19.09 [b]	36.62 [a]	13.01 [b]	32.10 [b]
09 AD IRI	44.83 [a]	31.40 [b]	39.51 [a]	30.66 [b]	62.06 [b]
11 AD IRI	42.15 [a]	27.04 [b]	39.26 [a]	31.34 [b]	58.39 [b]
05 AD IRI	34.78 [a]	29.06 [b]	39.99 [a]	39.72 [b]	68.78 [b]
06 AD IRI	35.48 [a]	28.95 [b]	37.13 [a]	41.93 [b]	70.88 [b]
01 AD iIRI	27.31 [a]	21.14 [b]	41.90 [a]	38.34 [b]	59.48 [b]
04 AD IRI	37.33 [a]	14.90 [b]	42.40 [a]	28.60 [b]	43.50 [b]
13 AD IRI	47.31 [a]	30.62 [b]	41.85 [a]	19.82 [b]	50.44 [b]
03 AD IRI	26.69 [a]	26.64 [b]	39.41 [a]	54.81 [a]	81.45 [a]
02 AD IRI	29.64 [a]	33.32 [b]	35.90 [a]	39.19 [b]	72.51 [b]
08 AD IRI	49.85 [a]	17.69 [b]	33.35 [a]	18.88 [b]	36.57 [b]
União	38.33 [a]	27.38 [b]	39.32 [a]	29.54 [b]	56.92 [b]
Pesagro Bord	33.13 [a]	28.96 [b]	33.97 [a]	19.20 [b]	48.16 [b]
Average	35.67	35.02	36.73	41.32	76.33
Standard Errors	0.56	1.21	0.32	1.36	2.25

[1/] Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability.

greatest coefficient of variation was found in number of tillers (NT); it was considered very high, reaching 30.39% in 2012 and 38.48% in 2013. In contrast, plant height (HGT) had low CV values in both years: 6.85 and 7.88% in 2012 and 2013, respectively. The stem diameter (SD) had very close coefficients of variation in the two years: 10.59 (2012) and 10.52% (2013), considered medium (Table 5). For leaf blade width (LW), the CV was high in 2012 (23.92%) and medium in 2013 (18.65%). The leaf-blade-length variable (LL) had a medium CV, ranging from 15.59 (2012) to 18.53% (2013) (Table 6).

Corroborating this study, other experiments with elephant grass have demonstrated that the NT variable, when compared with HGT and SD, was also the one with the highest coefficient of variation (Daher et al., 2004b; Rossi et al., 2010; Xia et al., 2010; Oliveira, 2013), and HGT showed the lowest CV (Daher et al., 2004b; Rossi et al., 2010).

Clustering of means of morpho-agronomic traits by the Scott-Knott test

The number of tillers in cut 1 varied from 137.00 in

Table 5. Summary of the variance analysis for number of tiller per linear meter (NT), plant height (HGT), stem diameter (SD), blade width (LW) and length (LL) of 85 genotypes of elephant grass.

Source of variability	DF	Mean squared									
		NT		HGT (cm)		SD (mm)		LW(cm)		LL (cm)	
		Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
Blocks	1	217.45	1609.25	0.13	15.63	87.96	308.77	0.67	0.00	1353.34	268.89
Treatments	84	1046.31**	1703.89**	0.39**	0.15**	8.64**	11.89**	0.82**	0.52**	167.05**	183.58**
Residue	84	3.352.70	7705.03	0.06	0.08	21.03	19.58	0.22	0.09	544.87	653.40
Mean		60.26	72.14	3.55	3.57	13.70	13.30	1.97	1.65	47.34	43.62
CV (%)		30.39	38.48	6.85	7.88	10.59	10.52	23.92	18.65	15.59	18.53

* -Significant at 1% probability by the F test; CV (%) - coefficient of variation.** - Significant at 1% probability by the F test; CV (%) - coefficient of variation.

Table 6. Number of tillers per linear meter (NT), plant height (HGT), stem diameter (SD) and leaf blade width (LW) and length (LL) in cuts 1 and 2, evaluated in 85 genotypes of elephant grass.

Genotypes	NT		HGT (m)		SD (mm)		LW (cm)		LL (cm)	
	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2	Cut 1	Cut 2
Elefante da Colômbia	55.00 b1/	47.00 b	3.70 b	4.40 a	13.41 c	13.56 c	2.17 c	1.48 c	42.30 c	37.92 b
Mercker	53.00 b	80.00 b	4.35 a	3.80 a	14.20 b	15.50 b	3.01 a	1.58 c	47.40 c	45.41 b
Três Rios	70.50 a	114.00 a	4.20 a	3.75 a	14.14 b	14.01 b	2.76 b	1.65 c	49.20 c	42.50 b
Napier Volta Grande	41.50 b	55.00 b	3.22 c	3.80 a	14.16 b	12.80 c	3.11 a	1.63 c	44.47 c	43.50 b
Mercker Santa Rita	73.00 a	70.50 b	3.25 c	3.70 a	11.88 c	13.38 c	2.64 b	1.48 c	37.50 c	42.17 b
Pusa Napier N 2	49.00 b	54.00 b	3.10 c	3.50 b	12.95 c	13.12 c	2.35 b	1.40 c	37.72 c	34.67 b
Gigante de Pinda	82.50 a	104.50 a	3.15 c	3.02 c	13.02 c	11.79 c	2.54 b	1.33 c	40.54 c	37.50 b
Napier N 2	42.50 b	60.00 b	3.17 c	3.27 b	11.14 c	12.06 c	2.55 b	1.48 c	41.65 c	40.66 b
Mercker S.E.A	48.00 b	59.00 b	3.27 c	3.40 b	10.84 c	11.37 d	2.23 b	1.71 c	35.95 c	40.33 b
Taiwan A-148	71.50 a	56.40 b	3.70 b	3.90 a	15.10 b	11.62 d	1.48 c	1.43 c	37.27 c	43.35 b
Porto Rico 534-B	52.00 b	87.50 a	3.80 b	4.05 a	11.89 c	13.41 c	1.40 c	1.55 c	43.25 c	46.91 b
Taiwan A-25	53.00 b	41.50 b	3.70 b	3.75 a	14.08 b	11.25 d	1.25 c	1.31 c	46.52 c	43.66 b
Albano	27.00 b	31.50 b	4.15 a	4.10 a	17.19 a	16.65 a	1.63 c	1.52 c	46.63 c	42.03 b
Hib, Gigante Colômbia	49.50 b	43.50 b	3.65 b	3.62 a	12.59 c	15.86 b	1.36 c	1.18 c	41.22 c	37.45 b
Pusa Gigante Napier	66.00 a	89.00 a	3.84 b	4.15 a	14.43 b	14.90 b	2.05 c	1.37 c	64.11 b	34.91 b
Elefante Híbrido 534-A	31.00 b	45.00 b	3.50 c	3.50 b	15.91 a	17.85 a	1.90 c	1.67 c	57.06 b	47.00 b
Costa Rica	30.00 b	45.50 b	3.97 b	4.05 a	16.06 a	17.07 a	2.01 c	1.57 c	48.98 c	44.58 b
Cubano Pinda	41.00 b	40.50 b	4.56 a	3.90 a	15.70 a	18.74 a	2.81 a	1.68 c	61.17 b	45.83 b
Mercker Pinda	42.00 b	35.00 b	3.31 c	3.65 a	13.26 c	18.09 a	2.21 b	1.26 c	44.43 c	40.08 b
Mercker Pinda México	36.50 b	36.50 b	3.32 c	3.75 a	15.36 b	19.29 a	1.50 c	1.25 c	44.96 c	37.66 b
Mercker 86 México	43.00 b	43.00 b	3.97 b	4.22 a	14.21 b	18.83 a	1.11 c	1.11 c	31.46 c	29.28 b
Taiwan A-144	69.00 a	77.50 b	3.90 b	3.85 a	13.83 b	14.20 b	1.02 c	1.36 c	42.83 c	33.17 b
Napier S.E.A	71.00 a	74.00 b	3.79 b	3.85 a	13.65 c	13.68 c	1.52 c	1.40 c	48.77 c	33.18 b
Taiwan A-143	50.00 b	50.00 b	4.05 a	3.75 a	14.54 b	12.67 c	1.35 c	1.20 c	47.08 c	32.38 b
Pusa Napier N 1	33.00 b	57.00 b	3.65 b	3.90 a	17.89 a	16.56 a	1.77 c	1.16 c	46.42 c	33.17 b
Elefante de Pinda	87.65 a	69.50 b	3.02 c	3.40 b	11.73 c	11.10 d	1.21 c	1.35 c	48.78 c	33.91 b
Mineiro	88.50 a	58.50 b	3.25 c	3.45 b	12.03 c	12.04 c	1.91 c	1.60 c	46.31 c	36.30 b
Mole de Volta Grande	112.50 a	119.00 a	3.42 c	3.45 b	13.06 c	9.88 d	1.76 c	1.30 c	49.16 c	38.08 b
Porto Rico	97.50 a	105.00 a	3.02 c	3.45 b	10.92 c	10.90 d	2.06 c	1.37 c	40.20 c	40.42 b
Napier	86.50 a	89.50 a	3.10 c	3.50 b	12.49 c	11.72 c	1.88 c	1.46 c	46.19 c	40.08 b
Mercker Comum	59.50 b	81.50 b	2.92 c	3.20 b	11.16 c	11.24 d	2.14 c	1.15 c	27.11 c	32.93 b
Teresopólis	80.00 a	91.50 a	3.73 b	3.80 a	11.70 c	12.57 c	1.28 c	0.95 c	31.90 c	21.90 b
Taiwan A-46	91.00 a	112.00 a	3.52 c	3.75 a	14.02 b	12.48 c	1.23 c	1.18 c	44.31 c	37.67 b
Duro de Volta Grande	68.00 a	92.50 a	3.75 b	3.70 a	12.65 c	11.96 c	1.74 c	1.43 c	46.00 c	43.05 b
Mercker Comum Pinda	89.00 a	71.00 b	3.10 c	3.40 b	12.80 c	11.14 d	1.73 c	1.06 c	33.01 c	32.33 b
Turrialba	56.00 b	72.50 b	3.77 b	3.60 a	15.74 a	15.42 b	1.25 c	1.66 c	49.02 c	42.36 b

Table 6. Contd.

Taiwan A-146	57.00 [b]	39.00 [b]	4.21 [a]	3.65 [a]	15.66 [a]	13.68 [c]	2.12 [c]	1.55 [c]	49.67 [c]	45.50 [b]
Cameroon – Piracicaba	61.50 [b]	89.00 [a]	4.01 [a]	3.75 [a]	16.40 [a]	15.90 [b]	1.85 [c]	1.93 [c]	55.74 [b]	56.87 [a]
Taiwan A-121	87.50 [a]	82.50 [b]	3.50 [c]	3.45 [b]	11.11 [c]	12.73 [c]	3.18 [a]	1.81 [c]	42.57 [c]	46.75 [b]
Vrukwona	41.00 [b]	101.00 [a]	4.15 [a]	3.70 [a]	17.63 [a]	13.38 [c]	1.47 [c]	3.17 [a]	54.46 [b]	59.92 [a]
P241 Piracicaba	50.20 [b]	98.00 [a]	3.42 [c]	3.85 [a]	13.67 [c]	13.14 [c]	3.46 [a]	1.60 [c]	57.60 [b]	56.03 [a]
IAC-Campinas	46.00 [b]	78.00 [b]	4.05 [a]	3.50 [b]	16.56 [a]	12.70 [c]	2.12 [c]	2.63 [b]	50.34 [c]	56.08 [a]
Elefante Cachoeira Itapemirim	137.00 [a]	54.50 [b]	3.30 [c]	3.65 [a]	12.25 [c]	12.94 [c]	3.30 [a]	1.28 [c]	49.34 [c]	28.71 [b]
Capim Cana D'África	45.00 [b]	132.50 [a]	3.95 [b]	3.70 [a]	16.95 [a]	12.77 [c]	2.10 [c]	2.31 [b]	51.08 [c]	54.50 [a]
Gramafante	83.00 [a]	42.00 [b]	3.30 [c]	3.50 [b]	12.30 [c]	11.29 [d]	2.86 [a]	1.71 [c]	48.60 [c]	39.53 [b]
Roxo	40.50 [b]	103.50 [a]	3.98 [b]	3.95 [a]	13.56 [c]	14.59 [b]	1.88 [c]	2.06 [b]	55.53 [b]	50.30 a
Guaco/I.Z.2	64.00 [b]	78.50 [b]	4.05 [a]	3.80 [a]	13.99 [b]	14.87 [b]	2.30 [b]	2.71 [b]	58.92 [b]	74.00 a
Cuba-115	37.50 [b]	39.00 [b]	3.95 [b]	3.60 [a]	14.86 [b]	14.16 [b]	2.22 [b]	2.10 [b]	50.82 [c]	46.65 [b]
Cuba-116	80.00 [a]	61.50 [b]	4.15 [a]	3.85 [a]	16.08 [a]	13.79 [c]	2.30 [b]	3.57 [a]	47.29 [c]	73.55 a
Cuba-169	31.65 [b]	47.50 [b]	3.30 [c]	3.20 [b]	16.14 [a]	15.21 [b]	3.30 [a]	2.40 [b]	68.26 [a]	60.50 a
King Grass	64.50 [b]	72.50 [b]	3.73 [b]	4.15 [a]	14.59 [b]	13.27 [c]	1.67 [c]	1.78 [c]	53.81 [b]	55.58 a
Roxo Botucatu	32.00 [b]	37.50 [b]	3.80 [b]	4.22 [a]	14.83 [b]	17.57 [a]	1.79 [c]	1.96 [c]	49.13 [c]	50.78 a
Mineirão IPEACO	75.00 [a]	101.00 [a]	3.45 [c]	3.60 [a]	11.01 [c]	11.73 [c]	1.72 [c]	1.61 [c]	44.47 [c]	40.58 [b]
Vruckwona Africano	53.50 [b]	73.50 [b]	3.32 [c]	3.60 [a]	13.38 [c]	10.62 [d]	1.47 [c]	1.90 [c]	50.26 [c]	46.50 [b]
Cameroon	41.00 [b]	52.00 [b]	4.23 [a]	3.60 [a]	17.73 [a]	13.85 [c]	2.87 [a]	2.33 [b]	45.97 [c]	54.58 a
CPAC	34.50 [b]	44.50 [b]	3.90 [b]	3.40 [b]	15.05 [b]	13.02 [c]	2.66 [b]	3.53 [a]	60.98 [b]	67.51 a
Guacu	43.00 [b]	55.00 [b]	3.45 [c]	3.55 [a]	13.91 [b]	14.24 [b]	2.30 [b]	2.25 [b]	78.24 [a]	60.83 a
Napierzinho	68.00 [a]	44.00 [b]	3.10 [c]	3.10 [b]	12.22 [c]	9.12 [d]	1.33 [c]	1.53 [c]	52.67 [b]	37.08 [b]
IJ 7125 cv EMPASC 308	88.50 [a]	105.00 [a]	2.97 [c]	3.45 [b]	12.19 [c]	12.03 [c]	1.45 [c]	1.55 [c]	40.12 [c]	46.13 [b]
IJ 7126 cv EMPASC 310	37.00 [b]	46.50 [b]	3.50 [c]	3.90 [a]	16.47 [a]	17.44 [a]	1.28 [c]	1.33 [c]	39.28 [c]	38.24 [b]
IJ 7127 cv EMPASC 309	67.00 [a]	157.00 [a]	3.25 [c]	3.00 [c]	9.80 [c]	10.75 [d]	1.42 [c]	1.10 [c]	45.46 [c]	35.50 [b]
IJ 7136 cv EMPASC 307	30.00 [b]	118.00 [a]	3.05 [c]	2.70 [c]	13.36 [c]	12.89 [c]	1.60 [c]	1.50 [c]	50.64 [c]	38.00 [b]
IJ 7139	51.50 [b]	64.00 [b]	4.29 [a]	3.25 [b]	18.58 [a]	14.72 [b]	3.26 [a]	2.56 [b]	42.56 [c]	55.66 a
IJ 7141 cv EMPASC 306	35.35 [b]	44.00 [b]	3.40 [c]	3.55 [a]	14.98 [b]	15.08 [b]	2.33 [b]	1.52 [c]	60.88 [b]	38.08 [b]
Goiano	38.00 [b]	62.00 [b]	3.60 [b]	3.40 [b]	15.33 [b]	15.06 [b]	2.30 [b]	1.48 [c]	57.29 [b]	46.50 [b]
CAC-262	95.00 [a]	81.50 [b]	3.47 [c]	3.75 [a]	12.27 [c]	11.07 [d]	1.72 [c]	1.53 [c]	48.07 [c]	41.83 [b]
Ibitinema	95.00 [a]	119.00 [a]	3.05 [c]	3.20 [b]	10.66 [c]	10.90 [d]	1.14 [c]	1.46 [c]	41.82 [c]	42.08 [b]
903-77 ou Australiano	121.00 [a]	168.50 [a]	3.22 [c]	3.70 [a]	11.21 [c]	13.28 [c]	1.81 [c]	1.30 [c]	43.59 [c]	38.25 [b]
13 AD	96.00 [a]	132.00 [a]	2.95 [c]	2.50 [d]	10.07 [c]	7.85 [d]	1.19 [c]	1.41 [c]	40.54 [c]	39.16 [b]
10 AD IRI	92.00 [a]	100.00 [a]	3.30 [c]	3.00 [c]	12.34 [c]	9.59 [d]	1.39 [c]	1.40 [c]	41.48 [c]	40.80 [b]
07 AD IRI	61.00 [b]	45.50 [b]	3.35 [c]	3.50 [b]	14.07 [b]	12.12 [c]	1.32 [c]	1.43 [c]	39.19 [c]	45.58 [b]
Pasto Panamá	52.00 [b]	73.50 [b]	4.42 [a]	4.25 [a]	15.38 [b]	15.61 [b]	1.86 [c]	2.02 [b]	47.86 [c]	54.08 a
BAG – 92	68.50 [a]	58.00 [b]	2.62 [c]	2.90 [c]	10.99 [c]	8.69 [d]	1.11 [c]	1.13 [c]	30.11 [c]	36.33 [b]
09 AD IRI	51.50 [b]	71.00 [b]	3.10 [c]	3.52 [b]	11.90 [c]	12.36 [c]	1.70 [c]	1.58 [c]	47.45 [c]	44.78 [b]
11 AD IRI	53.00 [b]	62.00 [b]	3.30 [c]	3.40 [b]	10.91 [c]	11.57 [d]	1.67 [c]	1.46 [c]	40.04 [c]	39.91 [b]
05 AD IRI	72.00 [a]	77.50 [b]	3.17 [c]	3.50 [b]	11.78 [c]	10.73 [d]	1.58 [c]	1.47 [c]	49.36 [c]	442.78 [b]
06 AD IRI	64.00 [b]	120.35 [a]	3.12 [c]	3.15 [b]	14.45 [b]	10.61 [d]	1.92 [c]	1.51 [c]	39.76 [c]	41.33 [b]
01 AD iIRI	79.15 [a]	70.00 [b]	2.92 [c]	3.30 [b]	11.50 [c]	14.41 [b]	2.26 [b]	1.47 [c]	46.75 [c]	29.17 [b]
04 AD IRI	40.00 [b]	76.25 [b]	3.05 [c]	2.35 [d]	12.45 [c]	11.34 [d]	1.54 [c]	1.23 [c]	38.62 [c]	37.75 [b]
13 AD IRI	57.50 [b]	50.00 [b]	2.75 [c]	2.35 [d]	9.97 [c]	9.20 [d]	0.96 [c]	1.16 [c]	43.37 [c]	36.91 [b]
03 AD IRI	51.50 [b]	54.50 [b]	4.12 [a]	4.00 [a]	16.35 [a]	15.64 [b]	2.15 [c]	1.67 [c]	50.41 [c]	48.91 a
02 AD IRI	39.50 [b]	54.50 [b]	4.31 [a]	4.25 [a]	15.07 [b]	15.23 [b]	1.93 [c]	1.28 [c]	55.69 [b]	42.33 [b]
08 AD IRI	43.50 [b]	59.00 [b]	2.85 [c]	3.40 [b]	11.28 [c]	9.94 [d]	1.72 [c]	1.60 [c]	35.31 [c]	44.43 [b]
União	36.50 [b]	41.00 [b]	3.61 [b]	3.45 [b]	16.73 [a]	13.50 [c]	3.30 [a]	2.53 [b]	73.95 [a]	58.58 a
Pesagro Bord	36.00 [b]	35.00 [b]	3.65 [b]	3.30 [b]	14.27 [b]	15.81 [b]	3.63 [a]	2.22 [b]	67.54 [a]	53.88 a
Average	60.26	72.13	3.55	3.57	13.69	13.30	1.97	1.65	47.34	43.62
Standard Errors	2.01	2.96	0.04	0.03	0.18	0.20	0.05	0.04	0.81	0.85

[1/] Means followed by the same letter in the column do not differ by the Scott-Knott test at 5% probability.

genotype Elefante Cachoeira Itapemirim to 27.00 in Albano, averaging 60.26 tillers per linear meter. In cut 2, the genotype with highest tiller production was Australiano, with 168.50 tillers. In contrast, the genotype with lowest tillering was also Albano, with 31.50 tillers. The average number of tillers in cut 2 was 72.14. Both cuts generated two different groups according to the Scott-Knott clustering at 5% probability (Table 6). Rossi (2010) analyzed fifty two genotypes of elephant grass at ten months of age and obtained an average of 44.5 tillers per meter, which is much lower than the average found in our study. Oliveira (2013) also found a much lower value, of 37.18 tillers per meter, evaluating seventy three genotypes of *P. purpureum* for six months. Several studies have also demonstrated a wide range regarding the number of tillers per linear meter. In the study of Oliveira (2013), the range was from 73 to 13 tillers, and Rossi (2010) reported from 102 to 17.2 tillers per meter. The analysis of the number of tillers is an important process, given that this trait is directly proportional to the potential of productivity of the genotype (Daher et al., 2004; Ribeiro et al., 2009). However, a high number of tillers is not always translated into high productivity; in an experiment with 17 genotypes of elephant grass and one hybrid with millet, Xia et al (2010) concluded that the group with the highest productivity had few tillers. According to Silva et al. (2009), tillering is also important because it provides greater soil cover and consequently a lower number of invasive plants. For the HGT variable, in cut 1, the average was 3.55 m, with Cubano Pinda being the tallest (4.56 m) and BAG-92, the shortest (2.62 m). The Scott-Knott clustering (P<0.05) generated three groups in cut 1. Four groups were generated in cut 2, and the tallest genotype reached 4.40 m (Elefante da Colombia), whereas the shortest reached 2.35 m (04 AD IRI), averaging 3.57 m (Table 6).

A similar value to that found in cuts 1 and 2 was reported by Oliveira (2013): 3.36 m, in 73 genotypes at six months of age. In this same study, the tallest genotype was Roxo Botucatu (3.75 m), and the shortest was 13 AD RI (2.78 m). Corroborating the present study, Oliveira et al. (2012) concluded that the genotype with the lowest height was Cubano Pinda, as compared with six other genotypes at seven months of growth. Kannika et al. (2011) also observed that at 12 months of age elephant grass can reach up to 5 m, which is similar to the average found herein. The importance of the plant-height variable in elephant grass is positively correlated with its dry matter yield (Daher et al., 2004; Xia et al., 2010). The results found by De Mello et al. (2002) agree with this assertion, because they found that irrespective of the season of the year, the selection of elephant-grass plants should be based on the dry matter yield, which is related to taller plants. The stem diameter of the plants in cut one averaged 13.70 mm (18.58 to 9.80 mm), with genotypes IJ 7139 and IJ 7127 cv EMPASC 309 showing outstanding values. However, in cut 2, the values varied from 19.29 to 7.85 mm (average 13.30 mm) for genotypes Mercker Pinda México and 13 AD, respectively (Table 6).

In the Scott-Knott mean clustering (P<0.05) for SD, cut 1 presented three groups, whereas four groups represented cut 2. Similar values to those of cuts 1 and 2 were obtained by Oliveira (2013): 12.32 mm, in the dry season, in an evaluation of 73 genotypes at six months old. Oliveira (2012) found the greatest diameter, 21.30, in Camerron-Piracicaba at seven months of age, and Oliveira (2013) mentions BAG-86 as the 6-mo-old genotype of greatest diameter, 22 mm. Both studies found greater diameter values compared with the present study.

De Mello et al. (2002); Daher et al. (2004b) and Xia et al. (2010) have demonstrated that the stem diameter is positively correlated with productivity. Nevertheless, it has also been reported that stem diameter has a negative correlation with the number of tillers, i.e., the number of tillers decreases as the stem becomes thicker (Italiano et al., 2006).

Regarding the LW trait, in cut 1 genotype Pesagro Bord had the highest value, 3.63 cm, whereas 13 AD IRI had the narrowest leaf, with 0.96 cm, and the average LW was 1.97 cm. For cut 2, the average was 1.65, in which genotype Cuba-116 had the highest value, 3.57, and Teresópolis had the lowest width, 0.95 cm. In this trait, both cuts 1 and 2 in the Scott-Knott mean clustering at 5% presented three distinct groups (Table 6). In genotypes at ages under that analyzed in this study, the average leaf-blade with for both rainy and dry seasons was higher than that described in the present study (De Mello et al., 2002; Schneider, 2013).

It should be stressed that leaf blade width is extremely important in the morpho-agronomic characterization of the elephant grass, because it has a high heritability (98%) in breeding programs with elephant-grass clones, and it is also not very affected by environmental changes (Silva et al., 2009).

The leaf blade length in cut 1, by the Scott-Knott mean clustering (P<0.05), generated three distinct groups, wherein the highest value was presented by Guacu (78.24 cm), and the lowest by Mercker Comum (27.11 cm), with an average of 47.34 cm. The Scott-Knott mean clustering at 5% divided cut 2 into two groups, in which the LL varied from 74.00 to 21.90 cm (average 43.62 cm), for the genotypes Guaco/I.Z.2 and Teresópolis, respectively (Table 6). Just as for LW, in leaf blade length, genotypes assessed at an age (two months) before that of the present study revealed higher values: 78.2 and 71.4 cm, for the rainy and dry seasons, respectively, with genotypes Napier SEA and Elefante da Colombia standing out in the rainy season and Elefante de Pinda in the dry season (De Mello et al., 2002). The leaf-blade length is positively correlated with dry matter yield, which demonstrates the importance of LL as a selection criterion in breeding programs (De Mello et al., 2002; Shimoya et al., 2002). Thus, it was possible to notice significant differences between the genotypes that had the highest and lowest measurements in the two periods assessed in the two years under study. The formation of groups in all variables, in the Scott-Knott clustering at 5%, demonstrates that there is a great differentiation of genotypes as to the studied traits (Oliveira, 2013).

There are other methods to evaluate diversity among genotypes, such as the isoenzyme analysis and the ISSR and RAPD markers. By the first analysis, Daher et al. (1997) and Freitas et al. (2000) demonstrated the genetic diversity among the evaluated elephant-grass accessions using isoenzymatic markers. And utilizing ISSR and RAPD markers, Lima et al. (2011) formed five and six groups in 46 genotypes of elephant grass by UPGMA. However, it should be pointed out that these types of analysis have higher costs; thus, in research with little capital available it is more advantageous to evaluate genetic diversity using morphological characters, which is also more practical and less time-demanding. Still, each method has its own importance, and it is preferable that a germplasm collection be as widely studied and characterized as possible so as to give greater support to research and to the collection database (Sudré et al., 2006).

Multivariate analyses

When the set of characters represents qualitatively different variables and there is no correlation among them the univariate variance analysis is the best procedure to be applied. However, when a dataset displays variables that are correlated, it should be assumed that there is multinormality and so a multivariate analysis of the variance should be carried out (Freitas et al., 2005).

For forages, it is indicated to perform a multivariate analysis in addition to the univariate analysis, given that the traits are usually correlated with each other, because they are measured at the same tussock. Moreover, they are expressed in different scales and units of measurement (Freitas et al., 2005).

Genetic divergence

To analyze the discriminatory power of the variables, it is necessary to evaluate their contribution together, using multivariate analyses (Cruz et al., 2004). Thus, in considering the number of genotypes, the assessed variables and the low discrimination between the genotypes, it is important to perform a multivariate analysis, utilizing canonic variables and dissimilarity (Mahalanobis distance) (Schneider, 2013).The accumulated variance of the first two variables corresponded to 64.6457% of the total variance, because much of it was diluted until the 9th principal component, corresponding to 98.4957% of all the variation available at the germplasm collection (Table 7).

According to Cruz and Regazzi (2001), in the first two canonic variables - the concentration of a great proportion of the total variance, in general referred to as above 80% - it is feasible to study the genetic diversity by geometric distances between parents on a scatterplot. However, Barros (1991) and Pereira et al. (1992) report that the distribution of the variance is associated with the nature and number of characters employed in the analysis, which is focused on the first components only when few characters of agronomic interest, or characters from the same group, are evaluated. Accumulated values below 80% in the first two principal components were also found by Daher et al. (1997), Shimoya et al. (2002) and de Oliveira et al. (2006), who obtained 43.94, 50.02 and 35.80%, respectively. By the analysis of the relative importance of the characters in the canonic variables (Table 8), it was found that the characters of least importance, that is, the discard characters, were NT2, with the highest weighting coefficient at CV10 (0.5395), LL2, with the highest weighting coefficient at CV9 (–0.7178), followed by SD1, having the highest weighting coefficient at CV8 (0.7543). LW2, however, was the character of greatest importance, having the highest weighting coefficient at CV1 and CV2 (0.9889 and –0.7770, respectively), followed by HGT2, with the greatest weighting coefficient at CV3 (–0.6814).

In this analysis, the characters of least importance are considered those relatively invariable or that have redundancy, that is, those are represented by other characters or by a combination of characters, in which the correlation is elevated (Cruz and Regazzi, 2001). Thus, the principal component of greatest importance is that which is the combination of the variables, explaining the greater proportion of the total variation of the data; the second, in turn, defines the next greater variation, and so on (Silva and Sbrissia, 2010).

Corroborating this study, Shimoya et al. (2002) observed that stem diameter and the length of the adult average leaf were the traits of least importance. Daher et al. (2000) also corroborates our study, describing the number of tillers per linear meter as a disposable variable. Daher et al. (1997 and 2000) found that among the most important traits that determine genetic divergence among the elephant-grass genotypes are plant height in the dry and rainy seasons and leaf blade width at the base of the third leaf, both agreeing with the present study.

The technique of principal components has the advantage of assessing the importance of each studied character on the total variation available among the studied genotypes, allowing for the discard of the least important, redundant characters because they are already correlated with other variables or because of their

Table 7. Estimates of the variances (eigenvalues, ëj) associated with the canonic variables and respective weighting coefficients (eigenvectors) of ten variables assessed in 85 elephant-grass genotypes.

ëj	Cumulative variance (%)	Variables									
		1	2	3	4	5	6	7	8	9	10
10.8412	50.1484	-0.2064	-0.0644	0.4949	0.1747	0.2378	0.2652	0.3709	0.5326	0.2011	-0.2989
3.1341	64.6457	-0.1336	-0.0496	0.3166	0.4907	-0.0472	0.5054	-0.3689	-0.4082	0.1467	0.2384
1.9232	73.5417	-0.4322	-0.0529	-0.3017	-0.3863	0.3888	0.5290	0.0900	-0.1439	-0.3321	-0.0048
1.3600	79.8326	-0.1779	0.3975	-0.0083	-0.3353	-0.0912	0.0675	0.2008	0.0409	0.5952	0.5373
1.1515	85.1589	0.6071	0.2384	0.1639	-0.0200	0.0976	0.2430	0.5505	-0.3971	-0.1308	-0.0300
0.9528	89.5662	-0.2978	0.1788	0.5825	-0.1086	0.0131	-0.3471	0.0563	-0.0823	-0.5350	0.3340
0.7343	92.9630	0.1170	0.4681	0.2831	-0.4320	-0.1084	0.1713	-0.4941	0.0168	0.0301	-0.4626
0.6388	95.9180	0.1903	-0.1672	0.1527	-0.1289	0.8042	-0.2837	-0.2465	-0.1555	0.2794	0.0753
0.5573	98.4957	-0.3503	-0.3021	0.1575	-0.1975	-0.2183	-0.2187	0.2446	-0.5588	0.3010	-0.4051
0.3252	100.0000	-0.3020	0.6368	-0.2634	0.4611	0.2566	-0.2297	0.0835	-0.1619	0.0257	-0.2624

1 - NT, cut 1; 2 - NT, cut 2 (per linear meter); 3 - HGT, cut 1; 4 - HGT, cut 2 (m); 5 - SD, cut 1; 6 - SD, cut 2 (mm); 7 - LW, cut 1; 8 - LW, cut 2 (cm); 9 - LL, cut 1; 10 - LL, cut 2 (cm).

Table 8. Relative importance of the characters in the canonic variables established by the linear correlation of ten standardized variables in 85 elephant-grass genotypes.

Canonic variable	Weighting associated with:									
	1	2	3	4	5	6	7	8	9	10
CV1	-0.1284	-0.3264	0.6958	0.0501	0.2806	0.2667	0.3462	0.9889	0.2496	-0.5296
CV2	-0.1282	0.1431	0.0590	0.4773	-0.0596	0.5150	-0.4359	-0.7770	0.1330	0.4224
CV3	-0.2010	-0.0598	-0.4534	-0.6814	0.5451	0.5681	0.1818	-0.1364	-0.3586	-0.0086
CV4	-0.5006	0.7014	0.1226	-0.2487	-0.2618	0.0179	0.0560	-0.7585	0.5858	0.9521
CV5	0.6658	0.1942	0.1516	-0.2515	0.1971	0.2694	0.6090	-0.3657	-0.1382	-0.0532
CV6	-0.4771	0.1896	0.4577	-0.1403	0.0476	-0.3458	0.2044	-0.5673	-0.6144	0.5917
CV7	-0.2073	0.5478	0.3100	-0.3576	-0.0728	0.1941	-0.5330	0.6974	0.0824	-0.8197
CV8	0.3616	-0.2236	0.0184	-0.1593	0.7543	-0.3079	-0.3385	-0.2770	0.2941	0.1333
CV9	-0.1343	-0.3729	0.3105	-0.1380	-0.2398	-0.2115	0.1635	0.0106	0.3691	-0.7178
CV10	-0.4720	0.5395	-0.3002	0.4301	0.2831	-0.2246	0.0763	0.2174	0.0561	-0.4648

1 - NT, cut 1; 2 - NT, cut 2 (per linear meter); 3 - HGT, cut 1; 4 - HGT, cut 2 (m); 5 - SD, cut 1; 6 - SD, cut 2 (mm); 7 - LW, cut 1; 8 - LW, cut 2 (cm); 9 - LL, cut 1; 10 - LL, cut 2 (cm).

invariance; this contributes to the reduction of labor, time and the cost expended on the trial (Daher et al., 1997; Cruz and Regazzi, 2001; Guedes et al., 2013).

In the analysis of the canonic variables, the genetic divergence was demonstrated by the two-dimensional scatter plot, which was determined by estimating the scores obtained from the two canonic variables of highest importance, with the

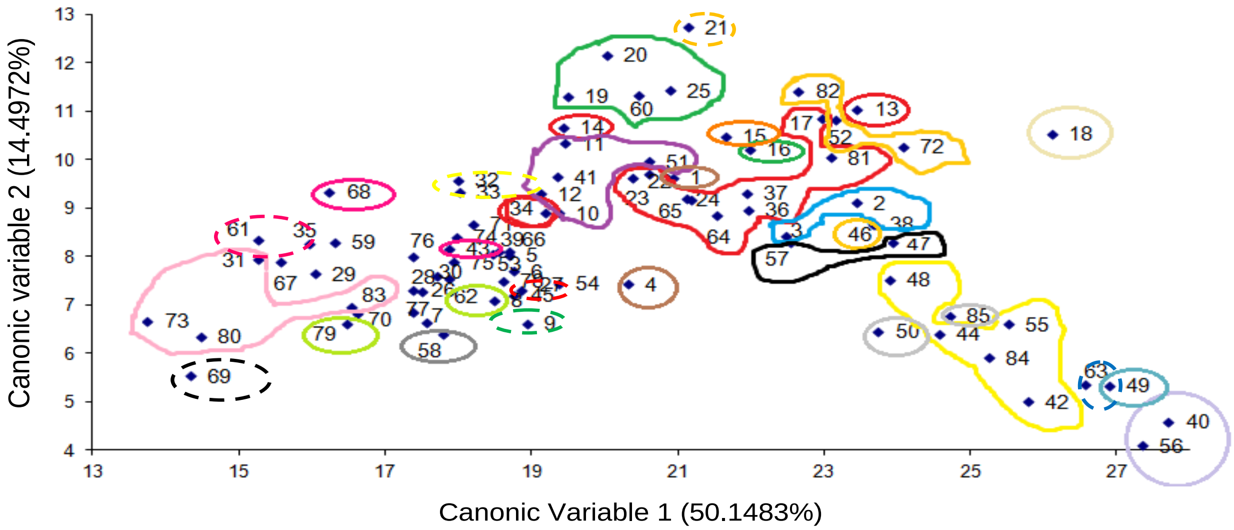

Figure 1. Scatter diagram of 85 genotypes of elephant grass, obtained by the analysis of the first and second canonical variables. Legend: G1 - red; G2 - no color; G3 - yellow; G4 - green; G5 - royal blue; G6 - purple; G7 - light grey; G8 - light pink; G9 - light orange; G10 - light green; G11 - lilac; G12 - dark pink; G13 - dark brown; G14 - black; G15- dark orange; G16 - beige; G17 - ocean blue; G18 - dark grey; G19 - dotted red; G20 - dotted orange; G21 - dotted green; G22 - dotted yellow; G23 - dotted blue; G24 - dotted pink; G25 - dotted black.

X axis being represented by the first canonic variable and the Y axis by the second canonic variable (Figure 1). The distribution of genotypes in the plot is a result of the means with the respective weighting coefficients established in each canonic variable.

The amplitude of the genetic distances coupled with the average of the mean distances of the 85 genotypes indicates a large genetic variability among the studied genotypes (Pereira et al., 2008). This divergence among genotypes was possible by the use of qualitative morpho-agronomic data and multivariate analysis (Sudré et al., 2006).

These results justify the use of analysis of canonic variables, because it simplifies the structure of the original data such that the divergence, at first influenced by a p-dimensional set, is represented in a two-dimensional space, with an easy geometric interpretation (Ferreira et al., 2003). This two-dimensional scattering enabled the separation of the genotypes into groups, and can be utilized as a strategy to select divergent groups to be utilized in future artificial crosses, aiming at genetic improvement (Neitzke et al., 2010).

Tocher's optimization method, shown in Table 9, agreed with the scattering of the genotypes in the two-dimensional graph (Figure 1), because the genotypes belonging to the same group remained close.The analysis of the clusters of the 85 elephant-grass genotypes by Tocher's optimization method based on Mahalanobis distance formed 25 divergent groups (Table 9). The mean distance within the group is the average of the distances between each pair of genotypes that compose it, and this distance is always smaller than the average intergroup distances (Cruz and Regazzi, 2001).

In the analysis, group 2 was composed of the largest number of genotypes, 22, and so the genotypes were subdivided into nine sub-groups. Eleven groups were generated with only one genotype in each one (Table 9).

The genotypes that make up the elite group, described previously (Porto Rico 534-B, Taiwan A-144, Napier S.E.A., Mole de Volta Grande, Teresópolis, Taiwan A-46, Duro de Volta Grande, Turrialba, Taiwan A-146, Cameroon- Piracicaba, Taiwan A-121, P241 Piracicaba, Elefante Cachoeira Itapemirim, Guaco/I.Z.2, Cameroon, IJ 7126 cv EMPASC 310, IJ 7139, Australiano, 10 AD IRI and Pasto Panamá), are in groups 6, 1, 1, 2, 22, 2, 1, 1, 1, 5, 2, 6, 12, 14, 3, 4, 23, 12, 2 and 9, respectively (Table 9). This indicates that possible crosses between pairs of genotypes belonging to different groups result in greater gains with heterosis, due to their dissimilarities. It should be emphasized that the objective of a breeding program is to increase productivity; thus, one should choose genotypes of satisfactory performance that were more divergent or that complement some trait of one of the parents (Ferreira et al., 2003; Guedes et al., 2013). Possible crossings between genotypes of the same group reduce the possibility of obtaining genotypes with different traits (Guedes et al., 2013).

Therefore, the non-involvement of individuals with the same dissimilarity pattern in crosses is suggested, so there will not be genetic variability and hence no negative impacts on the gains to be obtained for selection (Cruz and Regazzi, 2001). Studies on the genetic diversity of elephant grass utilizing Tocher's optimization demonstrate that this method has been well-used, providing a good perspective of the diversity among genotypes and information for future breeding programs based on

Table 9. Analysis of clustering by Tocher's optimization method obtained based on Mahalanobis distance (D²) for 85 genotypes of elephant grass.

Cluster	Subgroup	Genotypes											
1	-	22	23	24	34	37	36	81	17	65	64	14	13
2	2^{a}	39	53	45	66	76	30	75	74	26			
2	2^{b}	29	59	67	70								
2	2^{c}	89	65										
2	2^{d}	28	33										
2	2^{e}	35											
2	2^{f}	71											
2	2^{g}	27											
2	2^{h}	77											
2	2^{i}	54											
3	-	44	55	42	48	84							
4	-	25	60	20	19	16							
5	-	2	3	38									
6	-	10	12	51	11	41							
7	-	50	85										
8	-	31	73	83	80								
9	-	46	72	52	82								
10	-	62	79										
11	-	40	56										
12	-	43	68										
13	-	1	4										
14	-	47	57										
15	-	15											
16	-	18											
17	-	49											
18	-	58											
19	-	78											
20	-	21											
21	-	7											
22	-	32											
23	-	63											
24	-	61											
25	-	69											

hybridization (Daher et al., 2000, 2002; Shimoya et al., 2002; Pereira et al., 2008).

The number of groups formed by Tocher's method shows the large variability among the genotypes evaluated in this study, demonstrating their broad genetic diversity, which allows for the selection of different parents for breeding programs.

Conclusions

The genotypes that stood out for their dry matter yield were Porto Rico 534-B, Taiwan A-144, Napier S.E.A., Mole de Volta Grande, Teresópolis, Taiwan A-46, Duro de Volta Grande, Turrialba, Taiwan A-146, Cameroon - Piracicaba, Taiwan A-121, P241 Piracicaba, Elefante Cachoeira Itapemirim, Guaco/I.Z.2, Cameroon, IJ 7126 cv EMPASC 310, IJ 7139, Australiano, 10 AD IRI and Pasto Panamá, which composed the elite group. The cluster analysis provided the orientation for crosses involving ten heterotic groups, with the leaf blade width and plant height (cut 2) being the most important to explain the dispersion of genotypes. Tocher's optimization method, associated with Mahalanobis distance, allowed for the clustering of the eighty five genotypes of elephant grass belonging to BAG-CE into twenty five groups.

Conflicts of Interests

The authors have not declared any conflict of interests.

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Effect of Fipronil toxicity in haematological parameters in white leghorn cockerels

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Pesticide use in the modern agriculture, animal husbandry and public health practices has increased enormously. Pesticides affect man, environment and wild life including birds. Pesticides including fungicides, herbicides and insecticides are the major class of chemicals deliberately released into the environment because of their indiscriminate use in various agricultural practices. Pesticides are present in air, soil and water which leave their residue in food chain causing deleterious effect on the health status of man and animals including poultry. After ingestion, they get into blood stream lodging themselves in various tissues causing adverse effects on health and production of livestock and poultry resulting in substantial economic losses. Fipronil was discovered and developed by Rhone-Poulenc between 1985- 1987 and placed in the market in 1993. It is the member of new and relatively small class of pesticides, the phenyl pyrazoles, which are principally chemicals with an herbicidal effect. According to data obtained from metabolic studies showed that there was potential for bioaccumulation of the Fipronil in fatty tissues, fish, fibre crops and food crops. Limited investigations have been carried on effect of fipronil in health of animals and man. Therefore present study was designed to study the effect of fipronil toxicity in white leghorn cockerels. Twenty white leg horn male chickens of one month old weighing about 150 to 200 g were procured and kept at Instructional Poultry Farm Nagla, Pantnagar. The birds in poultry shed in deep litter system under standard managemental conditions. They were randomly divided into four groups of 5 birds each. After one week of adaptation period, different doses of Fipronil were administered orally through feed at 1, 5 and 10 ppm levels to groups G1, G2 and G3 respectively for 100 days daily. The birds of group "C" served as control. After every 20 days blood was taken from each bird for study of different parameters. The experiment was conducted for a period of 100 days. All the hematological parameters viz. hemoglobin, packed cell volume, total erythrocyte count and differential leucocyte count were performed on the day of blood collection as per the standard method. Hematological parameters showed significant decrease leading to anemia and significant decrease in total leucocyte count leading to suppression in cell mediated immune response in different doses of fipronil treated birds in comparison to birds of control group.

Key words: Fipronil toxicity, white leghorn cockerels, haematological parameters.

INTRODUCTION

Pesticide use in the modern agriculture, animal husbandry and public health practices has increased enormously. Pesticides affect man, environment and wild life including birds. According to Chauhan and Singhal (2006) 90,000 MT of technical grade pesticides are used annually to control pests and plant diseases in India. The pesticides are classified as insecticides, fungicides, weedicides, herbicides, nematodicides and rodenticides; of which insecticides constitutes 77% of the total pesticides used in different agricultural and animal husbandry practices and in public health operations. There is a gradual increase in production and consumption of pesticides during last few decades. The pesticides consumption increased from 2353 MT during 1955 to 75033 MT (technical grade) in the year 1991-1992 and which is again in the decline phase with the adoption of integrated pest management practices and the pesticides consumption level declined to the level of 43020 MT (technical grade) in the year 2003-2004. Pesticides are present in air, soil and water everywhere due to which they leave behind their residue in food chain (Ram et al., 1987; Kaushik et al., 1991) causing deleterious effect on the health status of man and animals including poultry. After ingestion, they get into blood stream lodging themselves in various tissues causing adverse effects on health and production of livestock and poultry resulting in substantial economic losses (Kaphalia and Seth, 1984). Chicken serve as good animal model for immunotoxicological risk assessment, that may be used for the determination of immune responses affected by environmental contaminants including pesticides (Linda et al., 1989). Moreover, poultry industry has made tremendous growth all over the world during the last decades. The poultry industry which was earlier classified as an unorganized sector has now turned itself as an organized sector through people, process and technology. According to Watt Executive Guide (2010) poultry industry has emerged as the most dynamic and fastest expanding segment in animal husbandry sector with 47.4 billion eggs produced by 2.4 billion layers and 3 billion broilers giving 2.25 million metric tons of poultry meat and likely to grow up to 75.6 billion eggs and 5.21 million metric tons of poultry meat by year 2012. The population of broiler and layer are 3 and 2.4 billion, respectively while per capita consumption of meat and eggs are 2.1 kg/annum and 42 eggs/annum respectively in India (Ravi, 2010) which is much less than ICMR recommendation against the requirement of 180 eggs and 10.8 kg of poultry meat (FAO, 2004). Reasons for more pesticide residue in India are indiscriminate and improportionate use of insecticide, lack of education and extension activity, inadequate literature supplied by manufacturers, want of more production and profit, lack of safer pesticides and use of banned pesticides. Keeping the above mentioned facts in mind studies on the toxicological impact of Fipronil on White Leghorn Cockerels (WLH) was planned.

MATERIALS AND METHODS

The present study was conducted to investigate haematological effect of induced Fipronil toxicity in white leghorn cockerels. Birds were continuously observed for different clinical manifestations throughout the trial from the beginning. Measurement of body weight of birds was taken after every 20 days interval in all groups till the end of trial.

Collection of blood samples

Blood samples for haematological examinations were collected from wing vein in sterilized disposable syringes (22 gauge needles) after proper restraining of birds. Blood samples were transferred to heparin coated tubes for haematological examinations. Collection of blood samples was done at the 20 days interval starting from day 0 during 100 days study. Approximately, 5 ml of blood per bird was collected. 125 IU/ml of blood heparin was used as anticoagulant for haematology.

One milliliter of blood was collected from each bird in clean heparin coated tubes and haematological parameters such as Total Erythrocyte Count (x 10^6/µl blood) and Total Leucocyte Count (x 10^3/µl blood) were determined according to the method of Natt and Herrick (1952) using poultry blood diluting fluid. Packed Cell Volume (PCV %) and haemoglobin (gm/dl) were estimated using the method of Jain (1986). Differential Leucocyte Count (DLC %) was done by preparing thin blood smear from a drop of blood without anticoagulant. The smear was air dried and stained with Leishman stained for 20 min. The leucocytes were counted by zig-zag method as described by Lucas and Jamroz (1961) and recorded on percent basis.

RESULTS

The results of induced toxicity of Fipronil in cockerels in the following blood parameters were as follows:

Packed cell volume (PCV)

The results of mean PCV values from different groups are presented in Table 1. There was significant (P<0.05) reduction in the PCV value in group G2 and G3 on as compared to the control on day 40,60, 80 and 100, whereas group G1 showed significant decrease as compared to the control after 60 days of feeding of the Fipronil.

Hemoglobin (Hb)

The results of mean Hb values from different groups are presented in Table 2. A significant (P<0.05) decrease was observed in group G1, G2 and G3 as compared to the control after 40,60 and 80 days of feeding of the Fipronil.

Total erythrocyte count (TEC)

The results of mean TEC values from different groups are

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Table 1. Mean packed cell volume (PCV %) in different groups of experimental birds (mean ± S.E).

Day \ Group	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	32.20±.382	32.21±.38	32.18±.383	32.22±.382
20 Day	32.21±.382	31.8±.382	31.68±.4	31.2±.38
40 Day	32.21±.382	31.5±.382	$30.2±.382^{wx,ab}$	$29.1±.382^{wx,ab}$
60 Day	32.20±.382	$29.76±.384^{w,abc}$	$29.4±.382^{w,ab}$	$28.1±.382^{wxy,ab}$
80 Day	32.15±.385	$28.4±.39^{w,abcd}$	$28.1±.382^{w,abcd}$	$27.33±.389^{w,abc}$
100 Day	32.21±.382	$27.96±.533^{w,abc}$	$27.5±.533^{w,abcd}$	$26.3±.382^{wx,abcd}$
Mean	32.20	30.27	29.84	29.04

a= Value differ significantly with column of 1^{st}row, b= Value differ significantly with column of 2^{nd} row, C= Value differ significantly with column of 3^{rd} row, d= Value differ significantly with column of 4^{rd} row, e = Value differ significantly with column of 5^{st}row, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Table 2. Mean hemoglobin concentration (gm/dl) in different groups of experimental birds (mean ± S.E).

Day \ Group	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	9.16±.212	9.16±.212	9.16±.212	9.16±.212
20 Day	9.17±.213	9.12±.212	8.98±.212	8.61±.212
40 Day	9.15±.212	8.96±.212	8.61±.212	$8.3±.212^{wx,a}$
60 Day	9.17±.213	8.6±.212	$8.24±.212^{w,ab}$	$8.03±.212^{w,a}$
80 Day	9.15±.212	$8.3±.21^{w,abc}$	$8±.212^{w,ab}$	$7.81±.212^{w,ab}$
100 Day	9.15±.212	$7.78±.123^{w,abcd}$	$7.9±.212^{w,abc}$	$7.6±.212^{w,abc}$
Mean	9.16	8.65	8.48	8.25

a= Value differ significantly with column of 1^{st}row, b= Value differ significantly with column of 2^{nd} row, C= Value differ significantly with column of 3^{rd} row, d= Value differ significantly with column of 4^{rd} row, e = Value differ significantly with column of 5^{st}row, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Table 3. Mean Total Erythrocyte Counts ($x10^6$ cells/ µl) in different groups of experimental birds (mean ± S.E).

Day \ Group	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	2.59±.035	2.59±.035	2.61±.035	2.58±.035
20 Day	2.57±.035	2.56±.035	2.56±.035	2.48±.035
40 Day	2.58±.035	2.54±.035	$2.43±.035^{wx,ab}$	$2.39±.035^{wx,a}$
60 Day	2.56±.035	$2.44±.035^{w,ab}$	$2.32±.035^{wx,abc}$	$2.31±.035^{wx,ab}$
80 Day	2.57±.035	$2.37±.035^{w,abc}$	$2.23±.035^{wx,abc}$	$2.16±.035^{wx,abcd}$
100 Day	2.58±.035	$2.25±.035^{w,abcd}$	$2.14±.035^{wx,abcd}$	$2±.035^{wxy,abcde}$
Mean	2.58	2.46	2.38	2.32

a= Value differ significantly with column of 1^{st}row, b= Value differ significantly with column of 2^{nd} row, C= Value differ significantly with column of 3^{rd} row, d= Value differ significantly with column of 4^{rd} row, e = Value differ significantly with column of 5^{st}row, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

presented in Table 3. There was no significant (P<0.05) change in among different treated groups on day 20 of the feeding trial, whereas after 40 days of feeding of Fipronil there was significant decrease in TEC values in group G2 and G3 as compared to the control. Significant decrease in G1 was observed after 60 days of feeding trial.

Total leucocyte count (TLC)

The results of mean TLC values from different groups are presented in Table 4. There was significant (P<0.05) decrease in the TLC in group G1, G2 and G3 as compared to control after 20 day of trial till the end of study.

Table 4. Mean Total Leucocyte Counts (x10^3 cells/ µl) in different groups of experimental birds (mean ± S.E).

Group / Day	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	24.56±.065	24.56±.087	24.56±.060^{wx}	24.55±.112^{wxy}
20 Day	24.56±.058	24.53±.027^{w,b}	24.3±.035^{wx,ab}	23.83±.035^{wxy,ab}
40 Day	24.55±.067	24.46±.035^{w,b}	24±.242^{wx,ab}	23.43±.035^{wxy,ab}
60 Day	24.57±.098	24.11±.035^{w,abc}	23.57±.098^{wx,abc}	22.77±.035^{wxy,abc}
80 Day	24.57±.070	23.61±.104^{w,abcd}	23.17±.053^{wx,abcd}	21.91±.035^{wxy,abcd}
100 Day	24.58±.137	23.13±.035^{w,abcde}	22.49±.035^{wx,abcde}	21.13±.035^{wxy,abcde}
Mean	24.56	24.07	23.68	22.94

a= Value differ significantly with column of 1strow, b= Value differ significantly with column of 2nd row, C= Value differ significantly with column of 3rd row, d= Value differ significantly with column of 4rd row, e = Value differ significantly with column of 5strow, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Table 5. Average values of Monocytes (%) different groups of experimental birds (mean ± S.E).

Group / Day	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	3.2±.2	3.2±.2	3.2±.2	3.20±.2^{a}
20 Day	3±.316	3.2±.2	3.4±.245	3.4±.245^{b}
40 Day	3.4±.245	3.2±.2	3.2±.374	3.2±.2
60 Day	3.2±.2	3.2±.316	3.2±.2	3.2±.2
80 Day	3±.316	3±.2	3±.316	3.4±.245^{e}
100 Day	3±.316	3.2±.2	3±.316	2.6±.245^{abe}
Mean	3.13	3.17	3.17	3.17

a= Value differ significantly with column of 1strow, b= Value differ significantly with column of 2nd row, C= Value differ significantly with column of 3rd row, d= Value differ significantly with column of 4rd row, e = Value differ significantly with column of 5strow, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Table 6. Average values of Lymphocytes (%) different groups of experimental birds (mean ± S.E).

Group / Day	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	65.6±.245	65.6±.245	64.8±.2^{wx}	65.40±.245
20 Day	65.6±.245	64.8±.372	63.8±.374^{w}	64±.316^{w}
40 Day	65.2±.2	64.6±.4	63±.316^{wx,a}	63.2±.8^{wx,a}
60 Day	65.2±.374	63.6±.51^{w,a}	63±.548^{w,a}	62.2±.49^{w,ab}
80 Day	65.8±.374	63±.55^{w,abc}	62±.447^{w,ab}	61.2±.583^{wx,abc}
100 Day	65.6±.245	62±.548^{w,abcd}	61±.316^{w,abcd}	60.2±.2^{wx,abcd}
Mean	65.5	63.93	62.93	62.7

a= Value differ significantly with column of 1strow, b= Value differ significantly with column of 2nd row, C= Value differ significantly with column of 3rd row, d= Value differ significantly with column of 4rd row, e = Value differ significantly with column of 5strow, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Differential leucocyte count (DLC)

Monocytes

The results of average monocyte values from different groups are presented in Table 5. There was a non significant change in all treated groups as compared to the control in the entire course of the study.

Lymphocytes

The results of average lymphocyte values from different groups are presented in Table 6. A significant ($P<0.05$) decrease was noticed in group G2 and G3 on day 20 and

Table 7. Average values of Eosinophils (%) different groups of experimental birds (mean ± S.E).

Group / Day	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	1.6±.4	1.6±.4	1.8±.374	1.6±.4
20 Day	1.6±.4	1.8±.374	2.2±.2	2.2±.2
40 Day	1.6±.4	2.2±.2	2.2±.2	2±.374
60 Day	1.8±.374	1.8±.2	2.2±.2	1.8±.2
80 Day	1.6±.4	1.8±.2	1.8±.374	2±.316
100 Day	1.6±.4	1.6±.374	1.8±.2	2±.316
Mean	1.63	1.80	2	1.93

a= Value differ significantly with column of 1st row, b= Value differ significantly with column of 2nd row, C= Value differ significantly with column of 3rd row, d= Value differ significantly with column of 4th row, e = Value differ significantly with column of 5st row, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Table 8. Average values of Heterophils (%) different groups of experimental birds (mean ± S.E).

Group / Day	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	29.6±.510	29.2±.583	29.8±.583	29.40±.510
20 Day	29.4±.510	29.8±.49^{w}	30±.894^{wx}	29.8±.583^{wx}
40 Day	29.4±.510	29.4±.51	31±.70 ^{wx,}	31±.632w^{x,ab}
60 Day	29.4±.510	30.8±.735^{a}	31.2±1.114	32.2±.374^{w,ab}
80 Day	29.2±.510	31.6±.4^{w,abc}	32.6±.245^{w,ab}	33±.316^{wx,abc}
100 Day	29.4±.510	32.6±.245^{w,abcd}	33.6±.4^{w,abcd}	34.4±.4^{wx,abcde}
Mean	29.4	30.57	31.37	31.63

a= Value differ significantly with column of 1st row, b= Value differ significantly with column of 2nd row, C= Value differ significantly with column of 3rd row, d= Value differ significantly with column of 4th row, e = Value differ significantly with column of 5st row, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

Table 9. Average values of Basophils (%) different groups of experimental birds (mean ± S.E).

Group / Day	C (0 ppm)	G1 (1ppm)	G2 (5 ppm)	G3 (10 ppm)
0 Day	0.6±.245	0.6±.245	0.6±.245	0.6±.245
20 Day	0.6±.245	0.4±.245	0.6±.245	0.6±.245
40 Day	0.6±.245	0.6±.245	0.6±.245	0.6±.245
60 Day	0.6±.245	0.6±.245	0.4±.245	0.6±.245
80 Day	0.6±.245	0.6±.245	0.6±.245	0.4±.245
100 Day	0.6±.245	0.6±.245	0.4±.245	0.8±.245
Mean	0.6	0.57	0.53	0.6

a= Value differ significantly with column of 1st row, b= Value differ significantly with column of 2nd row, C= Value differ significantly with column of 3rd row, d= Value differ significantly with column of 4th row, e = Value differ significantly with column of 5st row, w= value differs significantly with control group, x= value differs significantly with G1 group, y=value differs significantly with G2 group.

in all treated groups after 60 days onward as compared to the control group.

Eosinophils, Heterophils and Basophils

The results of average eosinophils, hetrophils, basophils values from different groups are presented in Tables 7, 8 and 9, respectively. There was a non significant change in eosinophils and basophils in all treated groups as compared to the control in the entire course of the study. A significant ($P<0.05$) increase in the heterophils was noticed in all the treated groups on day 80 and 100 as compared to the control.

A significant (P<0.05) decrease in Hb was observed in group G1, G2 and G3 as compared to the control after 40, 60 and 80 days of feeding of Fipronil respectively. There was no significant (P<0.05) change in TEC among different treated groups on day 20 of the feeding trial, whereas after 40 days of feeding of Fipronil there was significant decrease in TEC values in group G2 and G3 as compared to the control. Significant decrease in G1 was observed after 60 days of feeding trial. There was significant (P<0.05) decrease in the TLC in group G1, G2 and G3 as compared to control after 20 day of trial till the end of study. The decrease in Hb and PCV may be due to shifting of the fluid from extravascular compartment to the intravascular compartment in order to maintain the normal cardiac output and pooling of the blood cells in the blood reservoirs such as spleen etc. may contribute to fall in Hb, PCV, TEC and TLC. Also, these effects might be due to cytotoxic effect of insecticide. A significant (P<0.05) decrease in average lymphocyte % was noticed in group G2 and G3 on day 20 and in all treated groups after 60 days onward as compared to the control group. This might be due to cytotoxic effect of insecticide given in feed.

Aughton (1993) observed decrease in the erythrocyte parameters (Hb, PCV) in rats receiving 1.5, 30 and 300 ppm in diet in a combined oncogenic and toxicity study of 104 weeks.Holmes (1991b) reported decrease in Hb concentration in male rats and slight lower PCV, mean cell volume, mean cell Hb, prothrombin time in female rats given Fipronil at 300 ppm in the diet after 12 weeks in a 13 weeks study. According to report of FAO (1997) rats were given fipronil-desulfinyl by gavage for two weeks at doses of 0, 0.3, 1, 3, or 10 mg/kg bw per day. At 1 mg/kg bw/day, pale livers and reduced leukocyte counts were observed in females.

Conflict of Interest

The authors have not declared any conflict of interest.

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Comparative analysis of rice milling strategies: Evidence from rice millers in Benin

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Milling and related activities are central to the improvement of the quality of locally produced rice in many African countries. Fee-based milling and paddy buying-milling-selling services are among the strategies used in the milling business. The Heckman two-stage model and gross profit method were used on survey data, collected in 2011 on rice millers in major rice-producing regions of Benin to identify the factors that influence millers' choice of strategy and determine the quantity of paddy milled under the buying–milling–selling strategy. Results from the first stage of the Heckman model identified previous salary activity, utilization of modern technology, and trade as main activity as factors positively influencing millers' decision, while the second stage showed that the milling capacity and length of service in milling activity as factors positively influencing the quantity of paddy milled. The average profit of miller-traders was higher than that of millers-only.

Key words: Rice milling activities, determinants of paddy purchase, Heckman two-stage model, profitability.

INTRODUCTION

Since the 2008 food-price crisis, agricultural policy measures in many West African countries have focused on boosting domestic rice production. Consequently, West Africa's annual production of 7.7 MT is higher than that of any other region in Africa (AfricaRice, 2012). The consumption of rice is also growing in West Africa but rice processing is very fragmented with no major commercial players (Bill and Melinda Gates Foundation, 2012). Benin's Strategic Plan to Revitalize the Agriculture Sector (PSRSA, 2009–2015) includes the development of 13 promising sectors, including that of rice because of its socio-economic and dietary importance (NRDS, 2011). This has contributed to an increase in the annual growth rate of paddy production from 12% in 2000–2007 to 14% in 2008–2013 (USDA, 2014). This significant achievement should increase the volume of local rice available in Benin's major food markets. However, because of the low local rice processing capacity in the country, it is doubtful if the quality of such rice meets consumers' expectations. Locally produced African rice often fails to compete with imported rice because of outdated processing technologies and ill equipped infrastructure (Stryker, 2013; Seck et al., 2010, 2013). Most operators of rice hullers only provide milling services and this practice contributes to the fragmentation of the market for milled local rice (AfricaRice, 2011).

In Benin, the harvesting, threshing, drying and milling of rice are generally done manually and the end product is therefore often of low quality compared with imported rice. However, small threshing, winnowing and milling equipment have been introduced in some areas. Two

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processing methods are used - direct rice milling without parboiling and milling after parboiling. Rice milling without parboiling is common in the South while milling after parboiling is practiced in the North and Central zones. Parboiling itself is of two types, namely traditional parboiling and improved parboiling. Private millers in some villages use the Engelberg mill which produces grains of acceptable quality from parboiled rice. Processing is done by both men and women but, while women predominantly purchase, parboil and sell rice (usually through women's associations), milling is mostly done by men.

As mentioned earlier, rice produced in Benin is marginally less valued and attracts a lower price than imported rice due to perceived differences in quality. The low quality of local rice is also due to the status of the rice processing sector in which 76% of millers use traditional sub-standardized milling plants, 21% use small and medium-sized modern mills, and only 3% (in general the state mills) have automated plants that produce relatively good quality rice (AfricaRice and PAPA, 2011). Consequently, most of the locally processed rice contains impurities and mixed colors due to the use of inappropriate post-harvest handling such as post-milling sorting, polishing and grading. Much of this rice is consumed in rural areas (Demont, 2013). However, the product of mills that have key processing tools is clean and suitable for urban consumers who value convenience due to their busy work schedules (Lançon and David-Benz, 2007; Fiamohe et al., 2013). Rice processors have a major role to play in the improvement of the quality of local rice and their efficiency can be considered as key elements in the development of a new promising local rice market. According to Lançon (2003) the issue of the quality of locally produced rice is actually only relevant for miller-traders who have direct control over the type of paddy they process and bear the risks of selling a type of rice that does not respond to market demand; apart from specific paddy parboilers, parboiling is also done by millers who purchase paddy; most millers in Nigeria do not systematically combine milling with trading (that is, purchase paddy and sell milled rice) but rather mill paddy for a fee (that is, millers are service providers). Surveys of the rice-processing sector in Benin in 2011 showed that only 9% of millers combine milling with trading, 74% of millers are services providers, and 9% do both. This situation raises the following questions: (i) why can millers not purchase paddy and process it to a suitable standard for selling? (ii) which of these activities (providing only milling service or purchasing paddy to mill for sale) has positive effects on millers' welfare? (iii) which factors determine the economic attitude of millers and therefore the level of paddy purchased for milling and sale?

Astewel (2010) identified education level of the head of household and the quantity of rice produced as important variables that determine the volume of rice sold in the market by rice farmers. In contrast, the objective of Nigerian rice millers/traders is beyond maximizing the volume of rice processed; rather it is to generate additional income by adding marketing value to the product (Lancon, 2003). Through financial analysis Diarra (1994) showed that the buy–mill–sell activity is far more profitable than the provision of milling service. The many studies on rice post-harvest processing in Africa (Diarra, 1994; Lançon, 2003; Shwetha et al., 2010; Astewel, 2010) have ignored the factors that determine whether millers purchase paddy or not. This study assessed the factors that could explain the economic behavior of millers faced with the alternative strategies, and the quantity of paddy milled by those who adopt the buy–mill–sell strategy.

THEORETICAL FRAMEWORKS

Both descriptive methods and econometrics techniques were used. MCA provided an exploratory view of factors which explain how the rice milling sector in selected regions of Benin function. The Heckman two-stage model was then applied to determine the factors that influence millers' decision to buy–mill–sell and determine the quantity of paddy milled by miller-traders. Finally, profits of both categories of milling activities were calculated and compared.

Multiple correspondence analyses

MCA is a potentially useful exploratory data analysis technique for the identification of stable patterns in the data (Kaciak and Louviere, 1990). This technique was used to identify categories of millers that have similar characteristics in terms of criteria listed in Table 2. Normally, it consists of representing individuals or points (here millers or processing units) in a multidimensional space in such a way as to get an overview of the relative positions of individuals. However, it is difficult to observe points in a space with more than three dimensions. Therefore, MCA was used to reduce multidimensional space in a 3 orthogonal axes also termed as factors which maximize the information content (inertia). These axes define 2×2 factorial planes and are associated with eigenvalues. The eigenvalues are defined as the difference between the total number of modalities (K) of the variables and the total number of variables (Q) themselves divided by the total number of variables, [$(K-Q)/Q$].

Each modality of variables that characterize the economic behavior of millers is represented by a point. On the factor plane in the MCA points (projected points) that images of each other represent individuals that have similar or the same characteristics. Some criteria such as contribution of a modality to the total inertia borne by an

Table 1. Description of variables used for the MCA.

Type of variable	Variable	Definition of variable
	Hgender	Gender of processing unit's head
	EL	Education level
	SA	Secondary activity
	PA	Previous activity
	Techmach	Milling machine technology
Active variables	Modern machine	Using modern machine as technology
	Ordinary machine	Using ordinary machine as technology
	CatC	Category of customers
	CC	Existence of contract with customers
	TCC	Type of contract with customer
	SF	Source of financing
	LS	Length of service
Illustrative variables	PrAssM	Being member of processor association
	reasonMiRiT	Reason for milling rice
Continuous illustrative variables	Capacity	Milling machine capacity

axis (absolute contribution), good position of a modality on an axis (relative contribution) and appropriate modalities to use for the similarity interpretation of individuals on a given graphical display (test-value) are necessary. For instance, the higher the test-value of a modality on an axis, the more useful this modality is for interpreting that axis. Variables used in the MCA analysis are described in Table 1.

Heckman two-stage model

The micro-data for the quantity of paddy purchased and processed are available for only some millers since not all of them purchase paddy. Thus, we have zero quantity of paddy purchased for some millers. This situation created an econometric problem known in the literature as 'sample selection bias as specification error' (Heckman, 1979). To resolve this problem, we used Heckman's two-stage procedure. This procedure is applied when the data used are censored (Green, 2005). According to Gujarati (2003, pp. 563–636) a sample in which information on the regressand is available only for some observations is known as a 'censored sample'. This technique is appropriate because in this study we are not only interested in knowing factors that determine the economic attitude of millers, but also the level of paddy purchased and milled. If only the probability of a miller purchasing or not purchasing paddy were to be analyzed, Probit or Logit models would be adequate. Since we are interested in both objectives, there is a need for a model that is a hybrid between the Logit or Probit and ordinary least squares (OLS).

Sample selection bias may arise in practice for two reasons: (i) there may be self-selection by an individual or data units being investigated; (ii) sample selection decisions by analysts or data processors operate in much the same fashion as self-selection (Heckman, 1979). This method can also be used to address the issue of endogeneity. In that case, the endogeneity is supported by the presence of sample selection bias (Manuel, 2010). For this study, this model was chosen due to missing data for the regressand (quantity of purchased paddy). The Heckman method consists of a two-step estimating procedure. In the first stage, a 'selection equation' attempts to capture factors affecting millers' decision on the purchase of paddy. The second stage provides 'heckit' analysis that determines the quantity of paddy purchased and milled. The probability of millers purchasing paddy was modeled by the Maximum Likelihood Probit, from which the inverse Mill's ratio was estimated. Its specification is as follows:

The selection equation

$$Y_i = X_i\beta_i + \varepsilon_i \qquad (1)$$

$$\boldsymbol{Y_i} = \begin{cases} \mathbf{1}\boldsymbol{if}\ ,\ \boldsymbol{y_i^*} \succ \mathbf{O} \\ \mathbf{0}\boldsymbol{if}\ ,\ \boldsymbol{y_i^*} \leq \boldsymbol{C} \end{cases} \qquad (2)$$

where Y_i is a binary variable that assumes 1 if rice-processing unity (*i*) purchases paddy and 0 otherwise; β_i is the vector of unknown parameters to be estimated; X_i the vector of explanatory variables that determine whether paddy is purchased or not; ε_i is the random error terms that are assumed to be independently and

Table 2. Description of variables used in the model.

Variable	Type of variable	Description
First stage: Selection equation		
Paddy purchase	Endogenous	A variable that stands for rice millers' decision status as to whether they purchase paddy or not.
Previous salary activity	Exogenous	A dummy variable taking 1 if the miller had a previous salary activity and 0 otherwise.
Modern technology	Exogenous	A dummy variable taking 1 if a modern milling machine is used and 0 otherwise.
Trade as main activity	Exogenous	A dummy variable taking 1if the miller's main activity is trading and 0 otherwise.
Second stage: Regression equation		
Quantity of purchased paddy milled	Endogenous	A continuous variable that stands for the quantity of purchased paddy milled per day.
Machine capacity	Exogenous	A continuous variable that stands for the quantity of paddy a machine can mill per hour.
Length of service	Exogenous	A continuous variable that stands for the number of years the miller has spent so far in the activity.
Modern technology	Exogenous	A dummy variable taking 1 if a modern milling machine is used and 0 otherwise.

normally distributed with zero mean and constant variance; and y_i^* is the latent variable which is observed.

The regression equation (OLS)

$$Q_i = z_i\alpha_i + \mu\lambda_i + \eta_i \quad (3)$$

where Q_i is the purchased paddy milled; z_i the vector of explanatory variables which determine the quantity of paddy purchased and milled; α_i the vector of unknown parameters to be estimated and η_i the error term with the assumptions that:

$$\eta_i \rightarrow N(0,\sigma)$$

$$\varepsilon_i \rightarrow N(0,1)$$

$$corr(\eta_i, \varepsilon_i) = \rho$$

or $(\eta,\varepsilon) \rightarrow N(0,0,\sigma_\varepsilon^2,\sigma_\eta^2,\rho_{\varepsilon\eta})$.

Both error terms η *and* ε are normally distributed with mean 0, variances as indicated and the error terms are correlated where $\rho_{\varepsilon\eta}$ indicates the correlation coefficient. The term lambda(λ), which is related to the conditional probability that a rice-processing unit will decide to purchase paddy (given a set of independent variables) is determined by the following formula:

$$\lambda_i(-X_i\beta_i) = [\{\varphi(-X_i\beta_i)\ \}/\{1-\Phi(-X_i\beta_i)\}]$$

where φ and Φ are the probability density function and the cumulative distribution function of the distribution, respectively, and μ is a vector of unknown parameters that contains the correlation coefficient $\rho_{\eta\varepsilon}$. It is worth noting that *rho* (ρ) is the parameter that helps detect whether there is selection bias. For instance, if *rho* is statistically different from 0, there is selection bias and the two-stage model is relevant. Where *rho* is not statistically different from 0, OLS techniques will be appropriate.

Profit calculation of milling activities

We used the gross profit method to compute the profitability of rice milling for both categories of millers. The usual gross profit (GP) formula is specified as follows:

$$GP = V - C = PQ - \sum_i^n p_i q_i$$

where P is the price of the milled rice sold, Q the total quantity of milled rice sold, p_i the price of input *i* and q_i the quantity of input *i*. In the model, cost variables include repair and maintenance and depreciation costs (DC) of material (mills) which is specified as follows[1]:

$$DC = \frac{\text{(Initial cost) - (Salvage cost)}}{\text{Machine life}}$$

[1]Costs that are common for both types of activities include expenses on electricity, repairs, maintenance and labor.

Table 3. Regional distribution of surveyed millers.

Region	Frequency	Percentage (%)
Northwest	6	17.65
Northeast	14	41.18
Central	6	17.65
South	8	23.94
Total	34	100

The calculation of the value of production (V) and the cost of production (C) for each category of miller differed. For the miller-traders, the value of production was the market price per kg of milled rice multiplied by the quantity of milled rice. Also, costs incurred by the miller-traders took into account the value of paddy purchased (that is, quantity of paddy multiplied by the price per kg of paddy). On the other hand, the value of production for custom-millers was the fee per kg of milling service multiplied by the quantity of rice milled.

DESCRIPTIVE ANALYSIS OF SURVEYED DATA

Data collection

The data used in this study came from a survey conducted in 2011 across 16 communes in Benin by the national agricultural research system (Institut National des Recherches Agricoles du Benin, INRAB) and the national agricultural statistics systems (NASS) in collaboration with the Africa Rice Center.

This survey covered all actors across the rice value chain – farmers, consumers, traders and processors (millers and parboilers). In this study, we used only data that are relevant to rice millers since our goal was to investigate the factors that could explain the economic behavior of millers faced with alternative types of activities in Benin.

The survey used the same standard questionnaire and the stratified two-stage sampling method adopted by participating member states of AfricaRice. In the first stage, eight villages and peri-urban areas were purposively selected to ensure a good representation of the major rice processing zones with intensive milling activities. In the second stage, 90 processors were selected randomly in each village and Peri-urban area. However, because there are few millers in Benin, almost all of them were surveyed in each village and Peri-urban area and only 34 respondents were found. They fall into the different categories of small, medium and large-scale. Information collected with the structured questionnaire included detailed personal information of millers, sources of paddy procured, milling practices, milling costs, equipment, constraints, labor demand, labor skills, milling capacity, prices of local paddy and milled rice, energy sources, processing losses, and capacity utilization of the processing facilities. The geographical distribution of the surveyed millers is presented in Table 3.

Description of associated characteristics of surveyed population

Table 4 shows the socio-demographic characteristics of the surveyed millers. Of the 34 respondents, 82% were millers-only and 18% miller-traders; 94% were males and 6% females. Millers-only comprised 96% males and 4% females, compared to 83% males and 17% females among the miller-traders. This shows that the milling business in Benin is dominated by men.

Both groups of millers were ‘young’ with an average age of 28 to 50 years. More than 44% of millers in both categories were educated (4% of the millers-only group attended high school) while more than 40% were not educated. In addition, 33% of miller-traders used modern mills as against 14% in the millers-only group. The old types of mill were largely used by most millers in the two groups. The remaining variables were distributed more-or-less equally.

MAP OF MCA, ESTIMATED RESULTS FROM ECONOMETRICAL ANALYSIS AND PROFIT COMPUTATION

Map of rice millers

The map of millers with their respective characteristics is shown in Figure 1. The first factor axis explains 11.34% of the total variance, while the second axis explains 10.19%. The two factor axes explain a total of 21.53%. In the factor plane, the first axis is determined by the variable named contract with customers who have two modalities (contract with customers and no contract with customers). In the positive value is the modality contract with customers, (contribution=15.1, test-value=4.3, and distance=1.83) while in the negative value is the modality no contract with customers (contribution=8.2, test-value=–4.8, and distance=0.55). The second axis is determined by the variable funding source with two modalities (self-financing and donation/support). In the

Table 4. Socio-demographic characteristics of surveyed millers in Benin.

	Millers-only	Miller-traders
Gender	**%**	**%**
Male	96	83
Female	4	17
Age of the owner		
28–40	26	50
40–50	44	17
50–60	22	17
>60	7	17
Education		
Primary	41	33
Secondary	4	17
Higher	4	0
No level	22	17
Others	30	33
Source of financing		
Donation/support	37	33
Self-financing	63	67
Length of service		
<10 years	44	33
>10 years	56	67
Processors association members		
Member	67	67
Non-member	33	33
Previous activity		
General trade	12	17
Agriculture	50	50
Handicrafts	12	17
Salaried work	4	0
Workman	4	0
Others	19	17
Technology used		
Modern technology	15	33
Ordinary technology	85	67
Millers' Customers Category		
Association/group	0	17
Consumers	4	0
Enterprises	81	67
Producers	15	17
Public Institution	0	0
Contract with customers		
Yes	59	83
No	41	17
Type of contract with customers		
In-kind payment	27	100
Price discount	45	0
Short-term credit	18	0
Others	9	0

positive value is modality self-financing (contribution=7.2, test-value=–0.4, and distance=0.55) while in the negative value is the modality donation/support (contribution=13.3, test-value=0.4, and distance=0.83).

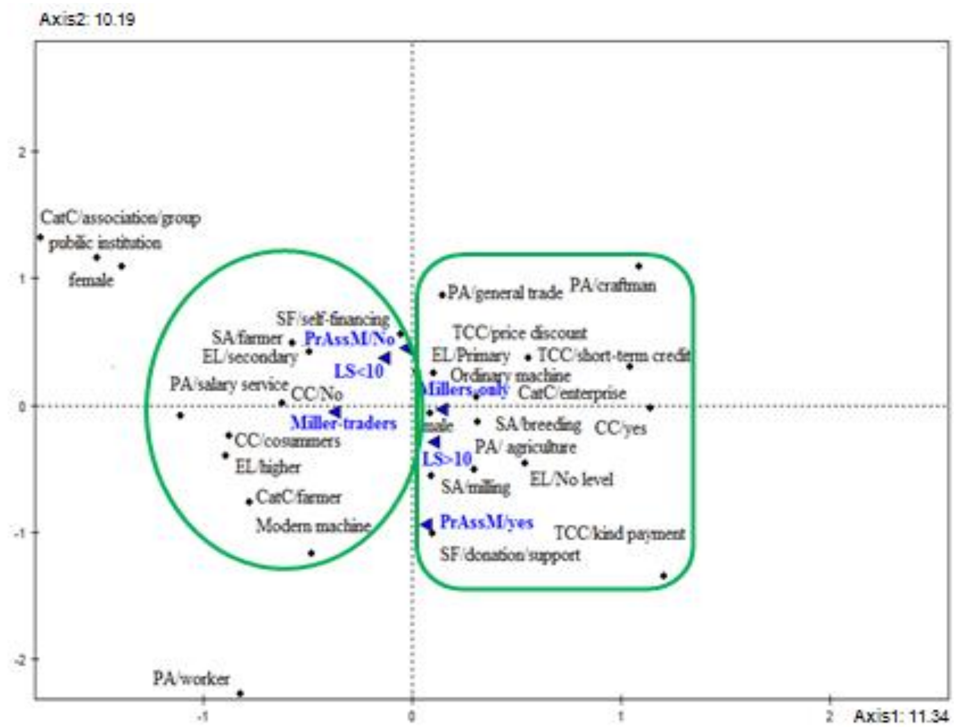

Figure 1. Map of rice millers with their distinctive characteristics.

The map reveals two main subgroups - miller-traders led by the modality no contract with customers and millers-only led by the modality contract with customers. Millers in the first subgroup are miller-traders, have less than 10 years of service (LS<10), do not belong to a processors' association (PrAssM/no), practice farming as a secondary activity (SA/farming), had salary service as a previous activity (PA/salary service), use more modern machinery, and have at least primary school education. The second subgroup comprises millers mill paddy as a fee-based service (Millers-only), belong to a processors' association (PrAssM/yes), have more than 10 years of service (LS>10), mill as a secondary activity (SA/milling), have general trading as a previous activity (PA/general trade), and use more old-fashioned machinery. Members of the second group often make short-term credit and in-kind payment contracts with their customers.

The exploratory analysis identified previous salary activity, the use of modern technology, length of service and trade as main activity as factors that are likely associated with the decision to purchase paddy or not. These results highlighted the similarity between the variables miller-traders (equivalent to purchase paddy) or millers-only (equivalent to not purchase paddy) and other characteristics of millers, such as the use of modern technology, previous salary activity, length of service, and trade as main activity. This apparent relationship between these variables is deepened by the econometrical analysis.

Results of the Heckman two-stage model

Table 5 presents results from the use of the Heckman two-stage model. This model explains factors that influence both the economic behavior of millers and the quantity of purchased paddy milled by them. The results show that the model is globally significant ($P<0.01$) and a *rho* figure that indicates whether there is statistically significant selection bias. The significance of *rho* confirms the appropriateness of the use of the Heckman two-stage model. On one hand, in the selection equation, the variables previous salary activity, modern technology and trade as main activity are statistically and positively significant ($P<0.01$). The significance of the previous salary activity suggests that millers who were previously in salaried employment had bought their mills using money they had saved. This is confirmed by the fact that on the MCA map, miller-traders bought mills by their own means. The significance of modern technology may be related to the fact that this technology is more used by miller-traders since they are the ones that purchased paddy. As for the variable trade as main activity, its significance can mean that millers that have trading as their main activity are more likely to purchase paddy and mill it for sale. Because paddy production is a seasonal activity, they diversify their activities by combining milling with trading. In Nigeria, 41% of all the millers closed their workshop for an average of three months because they were millers-only (Lançon, 2003). This fact highlights the

Table 5. Estimates from the Heckman two-stage model.

	Selection equation	
Exogenous variable	**Paddy purchase**	
	coefficients	**Marginal effects**
Previous salary activity	7.240*** (0.597)	1.689***(0.171)
Modern technology	6.956 ***(0.540)	1.623***(0.159)
Trade as main activity	7.359***(0.591)	0.170***(1.717)
Constant	0.108(0.284)	
	Regression equation	
Exogenous variables	Quantity of purchased paddy milled	
Machine capacity	0.297*** (0.014)	
Length of service	2.644*** (0.637)	
Modern technology	715.1213(868.097)	
Constant	887.852(933.55)	
rho	0.392*(0.238)	
Sigma	0.392***(0.238)	
Lambda	608.364***(324.695)	
Wald chi-squared (4)	4344.27	
Prob. > chi-squared	0.00	
Log likelihood	-231.293	
(*rho* = 0): chi-squared(1)	2.72	
Number of observations	34	

Standard errors in parentheses; *** P<0.01, ** P<0.05, * P<0.10.

difference between the two categories of millers.

On the other hand, the regression equation showed that the variables machine capacity and length of service in the milling business significantly and positively influence the quantity of paddy purchased and milled, while modern technology was not statistically significant. The significance of machine capacity means that the greater the capacity of a machine, the greater the quantity it mills when paddy is available. The significance of length of service could mean that over time, millers gain experience and acquire more skill and therefore their activity yields more. However, this is not corroborated by the MCA map, which indicates that length of service is associated with millers-only and is supposed to negatively influence the quantity of paddy purchased and milled since millers-only do not purchase paddy. This disparity can be explained by the fact that miller-traders are mainly those who entered the sector after liberalization, the length of service being more associated with millers-only (cooperative and public milling units which were established a long time ago) than miller-traders (more private sector operators who are newcomers to the sector). The non-significance of modern technology may be due to the fact that the difference between modern technology and ordinary technology does not lie in the quantity of paddy a machine can mill, but rather on the quality of the rice it produces. The estimated profit indicates the relative profitability of the two categories of milling activities and could be used by rice sector-oriented policy makers to offer financial-based advice.

Computation of profit

Table 6 shows the profit calculated for both types of millers, that is, those who purchase paddy (miller-traders) and those who do not (millers-only). The table describes the operating accounts of the two types of milling business as a simplified balance sheet providing each major cost item and source of revenue as well as the profit for each type of activity. The average profit for miller-traders for 1 kg was 35.465 FCFA compared with 15.98 FCFA for millers-only. Thus, milling was more profitable for miller-traders than for millers-only, others things being constant. It should be noted, however, that the profit of miller-traders is influenced by fluctuations in the price of paddy and milled rice since these millers are also involved in marketing.

Conclusions

Due to the inappropriate post-harvest techniques (threshing, drying, parboiling, and milling) used by processors, the quality of locally produced rice remains

Table 6. Operating accounts.

Processor / Item	Miller-traders	Millers-only
Quantity of parboiled rice (kg) (a)	11,600	41,476.57
Quantity of non-parboiled rice (kg) (b)	62,080	49,842.29
Total quantity (A = a+b)	73,680	91,318.86
Price of parboiled rice (FCFA kg^{-1}) (c)	346.41	NA
Price of non-parboiled rice (FCFA kg^{-1}) (d)	338.31	NA
Milling fee (FCFA kg^{-1}) (e)	NA	20
Total value for miller-traders [B=(a×c+b×d)]	25,429,807.3	–
Total value for millers-only [B=(a×e+b×e)]	–	1,826,377.2
Depreciation costs (FCFA) (f)	5,548.61	3,103.313
Repair and maintenance costs (FCFA) (g)	181,354.2	364,011.45
Expenditure on purchasing paddy (FCFA) (h)	22,220,868.8	NA
Total cost (f+g+h) (C)	22,407,771.61	367,114.77
Gross profit (total) (D =B–C)	2,613,077	1,459,262
Gross profit per kg (FCFA kg^{-1}) (E =D/A)	35.46	15.98

far below that expected by urban consumers. Processors thus have a major role to play in improving the quality of locally produced rice. This study assessed the factors that can explain the economic behavior of millers faced with the alternative types of activities - "providing only milling service for a fee" (millers-only) and "purchasing rice paddy and milling it for sale" (miller-traders). This was done by using multiple corresponding analysis (MCA) and the Heckman two-stage model. Whereas MCA facilitated the categorization of millers with the same characteristics, the Heckman two-stage model highlighted not only factors that influence millers' decision to purchase paddy or not, but also factors that influence the quantity of purchased paddy milled.

The MCA showed two subgroups of millers. Most of the miller-traders procured their mills with their own means; they are often not members of a processors' association; most of them have worked less than 10 years in the milling sector; they were previously in salaried employment, and some of them buy paddy and process it for sale. Most of the millers-only got their mills through support or donation; they are often members of a processors' association; do not purchase paddy to mill but process paddy for others for a fee (farmers, traders or consumers); most of them have spent more than 10 years in the sector and were previously involved in farming and handicrafts.

The Heckman two-stage model showed that previous salary activity, modern technology and trade as the main activity are likely to influence the decision of millers. It also showed that factors such as machine capacity and length of service influenced the quantity of purchased paddy milled. The average profit of miller-traders (35.46 FCFA kg^{-1}) is higher than that of millers-only (15.98 FCFA kg^{-1}).

These findings indicate that processors gain experience and acquire more skill over time. It is therefore, necessary to organize periodic training of processors, especially the newcomers. Policy should create an enabling environment by ensuring easy access to capital to facilitate investment in improved technologies by processors. Such policy could increase the number of miller-traders which is currently low. Millers also need a financial incentive to encourage them to purchase paddy. The expected outcome of this policy will be an increase in the use of modern mills that will produce good-quality rice as required by consumers.

Conflicts of Interests

The authors have not declared any conflict of interests

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Major postpartum problems of dairy cows managed in small and medium scale production systems in Wolaita Sodo, Ethiopia

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A longitudinal study was conducted on post-partum reproductive problems in small and medium scale dairy production systems in Wolaita Sodo from October 2011 to May 2012. The objectives of the study were to identify the major clinically manifested postpartum problems, estimating the prevalence and investigating the potential risk factors related to the occurrence of postpartum problems of dairy cows in the area. From a total 634 dairy cow examined, 57(8.99%) cows had different types of partum and postpartum problems: abortion (0.63%), dystocia (0.79%), retained fetal membrane (3.36%), uterine prolapse (0.47%), milk-fever (1.42%) and metritis (2.84%). A significant difference was observed in the occurrence of postpartum problems among age groups of cows (P <0.05) and parity (P <0.05). Higher prevalence of postpartum problems was recorded in older (15.20%, CI=7.82-22.61) than the younger cows. The prevalence of postpartum problems was higher in cows with seven and more than seven parity (15.70%, CI=7.11-24.31) than those cows having less than seven parity. Dairy cows postpartum problems were found to be one of the major problems in Wolaita Sodo. To reduce the postpartum problems due attention should be given for proper herd or individual cow management, early identification and diagnosis of the reproductive problems and awareness creation for owners.

Key words: Postpartum problems, dairy, risk factors, Wolaita Sodo, Ethiopia.

INTRODUCTION

The reproductive performance of zebu and improved cattle in tropical regions remains low, as the annual calving rate is about 50% (Bastidas et al., 1984). This low reproductive efficiency is due to various factors, and it becomes the major limitation to meat and milk production (Rodrfguez and Segura, 1995). Reproductive performance linked to the health of cows, which could be before and/or after calving. It has traditionally occupied a substantial amount of veterinarian's attention (DeVeries, 2006). Roelofs et al. (2010) concluded that long postpartum anestrus, poor estrus detection and low conception rates contributed to extending calving intervals in *Bos indicus* cattle. The postpartum period plays a pivotal role in cattle reproduction. The duration of postpartum anestrus has an important influence on reproductive performance (Lucy, 2007). Factors such as limited energy intake, lower body reserves, and postpartum diseases can potentially delay the return to cyclicity.

The 'Post-Partum Disease Complex' consists of

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ketosis, hypocalcaemia, metritis, retained fetal membranes, endometritis and uterine prolapse. All of these diseases are related to one another, with complicated cause and effect mechanisms in place. Numerous studies (Borsberry and Dobson, 1989; Gröhn et al., 1990; Rajala and Gröhn, 1998; Ferguson and Galligan, 2000; LeBlanc et al., 2002; LeBlanc, 2008) had shown that postpartum diseases can affect the length of calving interval, the number of days open, and the reproductive efficiency in general. These diseases can also affect the overall productivity of dairy cows by reducing milk yield.

Studies conducted so far in Ethiopia (Negussie et al., 1998; Shiferaw et al., 2003; Asseged and Birhanu, 2004; Nuraddis et al., 2011; Dinka, 2012) revealed poor reproductive performance of dairy cows in the tropics. For feasible intervention, the poor reproductive performance of dairy cows should warrant investigation on the types and magnitudes of the existing postpartum problems.

Therefore, the present study was conducted to identify and estimate the prevalence of the major clinically manifested postpartum problems and to see the effect of some risk factors in the occurrence of postpartum problems in dairy cow in and around Wolaita Sodo.

MATERIALS AND METHODS

Study area

The study was conducted in Wolaita Sodo, Southern Ethiopia, which is located at latitude of 6°54″N and 37°45″E. The altitude of the area ranges from 1,600 to 2,100 m above sea level. The study area is characterized by bimodal pattern of rainfall, and has an annual rain fall ranging from 450 to 1446 mm. The mean annual temperature of the area is about 10°C. The average maximum monthly temperature (18°C) is obtained in January and February while average minimum monthly temperature (11°C) is recorded in June and July (http://en.wikipedia.org/wiki/Sodo). The total cattle population of Wolaita Zone is estimated to be 723,343; of which 290,836 are 3 to 10 years old and 22,479 are older than 10 years (CSA, 2012).

Study population

The study population was dairy cows found in and around Wolaita Sodo with more than four months of pregnancy. Hence, they were calved in the study period, November 2011 to May 2012. A longitudinal study method was employed in which all selected cows were followed up to their parturition as well as during postpartum period. Reproductive history of study cows were collected carefully assessed and recorded, from record or owners'. Maximum effort was made to include all the pregnant, more than four months of gestation periods. At the beginning of the study, 634 cows were selected and registered, and then followed-up during their pre-partum and on their postpartum periods.

Study methodology

All selected cows were given code number for ease of identification. Then, all the necessary informations: breed, age, parity, body conditions, management system, methods of service, date of service, date of calving, and reproductive health conditions before and after calving were recorded. Age of the study animals was estimated from the record as well as dentition (De-Lahunta and Habel, 1986), and accordingly, the cows were grouped as young adults (3 to 6 years), adults (6 to 10 years) and old (greater than 10 years). The parity was classified as few (with less than or equal to 3 calves), moderate (3 to 7 calves) and many (greater than or equal to 7 calves). The body condition score were estimated as described by Nicholson and Butterworth (1986) and finally, three categories were used: poor, medium and good. The breeds of the animals were identified based on phenotypically, history from owners and the available record. The management system was classified into good, medium and poor (based up on husbandry variables; management and labor force, record keeping, housing, presence of calving pen, floor type, drainage, and general farm hygiene). The study cows and/or farm were visited on weekly basis both before and after parturition. Moreover, maximum effort was made to follow-up clinical cases and the process of parturition through arranged call from the farm owners. Any abnormality or terminal event that include live birth, dystocia, still birth, uterine prolapse, metritis, hypocalcaemia others were recorded.

Case definition

Dystocia: Dystocia was determined based on the history of occurrence from the farmer and clinical examination of the dam that requires calving assistance.

Metritis: This was determined based on the history of occurrence, general examination for the presence of fetid vaginal discharges, straining by the cow, inflammation and oedema of the vulva and rectal examination of the uterus for its size and contents.

Milk fever: This was determined based on history of occurrence and clinical signs such as reduced rectal temperature, depressed mental state, dry muzzle, weak pulse and heart sounds, generalized muscle weakness, ruminal atony and tympany, dilation of pupils with slow pupillary light reflex and recumbency.

Retained placenta: This was determined based on the history of a placenta that had not dropped within 12 h after calving and observation of the placenta hanging outside the vaginal opening or physical palpation per-vaginum.

Abortion: This was determined based on observation and history of termination of pregnancy before the full term.

Uterine prolapse: This was determined based on the history of occurrence from the farmer and clinical observation of the organ hanging outside the vulva opening.

Data management and analysis

Collected data entered and stored in spread sheet of Microsoft Office Excel. Then, it was summarized by means of descriptive statistics. The χ^2 test was employed for analysis of the various risk factors association with PPP. For this analysis, STATA software version 11, Stata Corp. 4905 Lake way drive College Station, Texas 77845, USA was used. The analysis considered the confidence level of 95% and $P<0.05$ was set for establishing significance.

RESULTS

Occurrence and prevalence of postpartum problems

Of the total 634 dairy cows examined 57 (9.0%) of them

Table 1. Reproductive problems of dairy cows encountered during the study period (n=634).

Reproductive/metabolic problems	Frequency	Proportion (%)	95% CI
Abortion	4	7.0	0.3-13.7
Dystocia	5	8.8	1.3-16.2
Retained fetal membrane	23	40.4	27.5-53.2*
Uterine prolapse	3	5.3	0.1-1.11.1
Milk fever	9	15.8	6.2-25.4
Metritis	18	31.6	19.4-43.8*

* = Significantly different, χ^2 = 589.8665 P < 0.05.

Table 2. Prevalence of PPP in calving cows with the different putative risk factors.

Risk factors	Number of cows examined	PPP No. (%)	95% CI	χ^2	P-value
Breed					
Local	386	23 (5.95)	3.59 - 8.33	3.66	0.056
Cross and exotic	248	25(10.1)	6.32 - 13.84		
Age					
Young adult	227	8(3.5)	1.12 - 5.93	13.21	0.001
Adult	315	26(8.3)	5.20 - 11.30		
Old	92	14(15.2)	7.82 - 22.61		
Body condition					
Good	256	25 (9.0%)	5.47 - 12.5	4.36	0.11
Medium	310	24(7.70)	4.76 - 10.73		
Poor	68	1(1.50)	1.42 - 4.36		
Method of mating					
AI	580	45(7.80)	5.58 - 9.94	0.34	0.56
Natural (bull)	54	3(5.60)	0.62 - 11.73		
Parity					
≤ 3	308	11(3.60)	1.49 - 5.65	16.12	0.000
3-7	256	26(10.20)	6.44 - 13.87		
≥ 7	70	11(15.70)	7.11 - 24.31		
Management					
Good	238	21(8.80)	5.21 - 12.44	1.23	0.54
Medium	335	24(7.20)	4.39 - 9.93		
Poor	61	3(4.90)	0.56 - 10.40		

PPP = Post-partum problems, CI = Confidence Interval.

were affected by various types of reproductive problems (Table 1). Five (0.8%) cows were affected with more than types of postpartum problems.

Risk factors analysis for postpartum problems

The prevalence and analysis of postpartum problems among breed, age, body condition, methods of mating, parity and management system were shown in Table 2.

The effect of the considered risk factor on the common postpartum reproductive problems was summarized, and shown in Table 3.

DISCUSSION

The finding of this study showed that more postpartum

Table 3. Analysis of reproductive problems in cows vs. putative risk factors.

Risk factors	No. of cows examined	Abortion No. (%)	Dystocia No. (%)	RFM No. (%)	UP No. (%)	MF No. (%)	Metritis No. (%)
Breed							
Local	386	3(0. 8)	4(1.0)	9(2.3)	1(0.3)	3(0.8)	11(2.9)
Cross and exotic	248	1(0.4)	1(0.4)	14(5.6)*	2(0.8)	6(2.4)	7(2.8)
Age							
Young adult	227	0	5(2.2)*	4(1.8)	1(0.4)	0	4(1.8)*
Adult	315	3(1.0)	0	13(4.1)	1(0.3)	4(1.3)**	10(3.2)
Old	92	1(1.1)	0	6(6.5)	1(1.1)	5(5.4)	4(4.4)
Body condition							
Good	256	2(0.8)	3(1.2)	10(3.9)	3(1.2)	5(2.0)	7(2.7)
Medium	310	2(0.7)	2(0.7)	12(3.9)	0	4(1.3)	1(3.6)
Poor	68	0	0	1(1.5)	0	0	0
Mating method							
AI	580	2(0.4)**	4(0.7)	21(3.6)	3(0.5)	9(1.6)	16(2.8)
Natural(bull)	54	2(3.7)	1(1.9)	2(3.7)	0	0	2(3.7)
Parity							
≤ 3	308	3(1.0)	5(1.6)	6(2.0)	1(0.3)	0	6(2.0)
3-7	256	0	0	12(4.7)	2(0.8)	4(1.6)**	9(3.5)
≥ 7	70	1(1.4)	0	5(7.1)	0	5(7.1)	3(4.3)
Management							
Good	238	2(0.8)	2(0.8)	10(4.2)	2(0.8)	5(2.1)	6(2.5)
Medium	335	2(0.6)	3(0.9)	11(3.3)	1(0.3)	3(0.9)	11(3.3)
Poor	61	0	0	2(3.3)	0	1(1.6)	1(1.6)

**Indicates highly significant difference ($P < 0.01$), * Indicates significant different ($P < 0.05$), BC = body condition, RFM = Retained fetal membrane, UP = Uterine prolapse, MF = Milk fever.

problems occurred in older cows (15.2%, CI=7.8-22.6) than the younger. This finding is in agreement with Thompson et al. (1983) who reported higher chance of postpartum problems in lactating old age cows and also increased with parity. According to Kahn and Line (2005) and Halpern et al. (1985) older cows have higher prevalence of parturient paresis, and are also at increased risk of retained placenta and metritis. These problems could be associated with reduced defense mechanisms in older and multiparous cows compared to younger cows. Moreover, older cows especially those that gave birth to many calves were exposed to repeated traumatic damage of reproductive tract, and hence, have poorly regenerating endometrium (LeBlanc, 2008; Ball and Peters, 2004). During this study, retained fetal membrane and metritis were more frequently observed. The problems were reported from various parts of the country (Bitew and Prasad, 2011; Lemma and Kebede, 2011) as one of the major postpartum problems in dairy cows. Retained fetal membrane (40.4%) was the most prevalent postpartum problem observed in the study area (Table 1). This problem was higher in older cows (6.5%) and followed by adult (4.1%), and young adult cows (1.8%). Similarly, Cows with more than or equal to seven parities were more frequently affected (7.1%) than the others. This observation was in agreement with Erb and Martin (1980) who reported that fetal membrane retention increased with advancing of parity or aging of cows. Ferguson and Galligan (2000) also reported about 10%, ranging from 6.3 to 14.6%, incidence rate of retained placenta in older cows. This problem, retained placenta can predispose to impairment of subsequent reproductive performance (Ball and Peters, 2004). Tefera et al. (2001) reported that placental retention was followed by significantly increased periods of abnormal vaginal discharge, intervals to uterine involution, intervals to first ovulation and rates of endometritis as compared with cows that were not affected. In addition, there is an increased frequency of abortions during subsequent pregnancies, possibly as a result of scar tissue formation within the uterine wall limiting expansion of the uterus and/or nutrition of the fetus (Noakes et al., 2001). The prevalence of metritis, the second (Table 1) most common post partum problem (2.8%), which was significantly (P <0.05) was influenced by age and parity (Table 3). Higher prevalence of metritis was observed in older cows (4.4%) and cows with more than or equal to seven parity (4.3%). Erb and Martin (1980) reported that older cows were 1.6 to 2.5 times as likely to develop metritis as were younger cows. Even though several factors are implicated as predisposing to reproductive tract infection, retained placenta and dystocia result in a greater incidence of reproductive tract infections (LeBlanc, 2008; Yoseph et al., 2005). According to Abuom et al. (2012) cows that developed retained placenta and dystocia were 5.2 and 3.9 times more likely to develop metritis, respectively. Therefore, retained fetal membranes was an important risk factor for vulval discharge (Peeler et al., 1994) and increased risk of metritis (Könyves et al., 2009), which can in turn influence the reproductive performance. Milk fever was the third prevalent (1.4%) postpartum problems encountered during this study period in Wolaita Sodo (Table 1). This problem could be associated with the ration particularly in minerals. Reist et al. (2003) reported that raised milk acetone concentrations (>0.40 mmol/L) were associated with endometritis. This suggests that metabolic disorders like milk fever and ketosis may predispose to, or be associated with, the uterine infection, and also provides a possible means of identifying cows at risk, so that appropriate interventions can be considered.

Postpartum problems were more prevalent in exotic and crosses, with greater than or equal to 50% exotic blood, cows. The higher prevalence observed in improved breeds could be associated with the lower degree of adaptation to the existing climate and poor management practice. Moreover, stress, the inability of cows to cope up with its environment, can predispose them to postpartum problems, and hence, they fail to achieve their genetic potential (Dobson and Smith, 2000). It is quite clear that poor metabolic status, negative energy balance in particular, can predispose to some postpartum problems (Ball and Peters, 2004).

The prevalence of dystocia was significantly higher (P <0.05) in young adult cows (Table 3). Though dystocia has maternal and fetal causes, the most common causes of dystocia are fetal in origin and these are invariably due to either fetal oversize or abnormal disposition of the fetus (Ball and Peters, 2004). Premature breeding in young cows, the common feto-pelvic disproportion in local cows mated with improved sire, and the increased fat around the perineum of over fed cows could be the possible reasons (Hafez and Hafez, 2006; Noakes et al., 2001).

Abortion was another reproductive problem in Wolaita Sodo, which was about 10 times more frequent in naturally mated cows (3.7%) than the artificially inseminated cows (0.4%).

Generally all the postpartum problems observed in the study area have great influence on productivity of the cows. According to Rajala and Gröhn (1998) and Lucey et al. (1986), retained placenta had a significant negative effect on milk yield for several weeks after calving. There is a considerable milk loss as a result of difficult of calving (Dematawewa and Berger, 1997). Metritis result in decreased dry matter intake, and hence, multiparous cows with metritis in early lactation produce less milk than the healthy cows. This difference is greatest during the first 20 weeks of lactation (Wittrock et al., 2011).

Conclusions

This study indicated that retained fetal membrane, metritis and milk fever were the most important reproductive problems in the study area. Age, breed, parity and mating methods were the most important predisposing risk factors for the various reproductive problems. The higher prevalence of postpartum problems can mainly attribute to the age and breed of cows in the study area. Postpartum problems can lead to heavy economic losses through reduced milk production (that is, by reducing the quality and quantity of milk), increasing veterinary expenses and poor reproductive performance (Fertility of such cows often reduced in terms of calving interval, calving to conception interval and pregnancy rate to first insemination). Therefore, owners' awareness creation and preventive measures for postpartum reproductive health problems should be instituted.

Conflict of Interest

The authors have not declared any conflict of interest.

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Effect of precision land levelling and permanent raised bed planting on soil properties, input use efficiency, productivity and profitability under maize (*Zea mays*) – wheat (*Triticum aestivum*) cropping system

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Precision land leveling with permanent raised bed planting with recommended dose of NPK can be used to improve crop yield, water and nutrient use efficiency over the existing traditional land leveling with flat beds planting with recommended dose of NPK practices. The objective of the present study was to establish an understanding of maize-wheat rotation yield and input use efficiency can be improved and how land leveling and crop establishment practices can be modified to be more efficient in water use through precision conservation crop management techniques. To conduct a farmers participatory field experiment during 2009-2011 in the jurisdiction of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P. Multi crop planter with inclined plate seed metering device machine were given to the farmers and crops were sown on permanent raised beds in maize-wheat cropping system. The data collected from the farmers participatory field experiment showed that there was about 20.4% (295.8 mm/ha for wide beds that is, 107 cm furrow centre gap) water saving and about 16.5% (310.3 mm/ha for narrow beds that is, 37 cm furrow centre gap) with grain yield increase of about 13.5% (5.13 and 4.44 t/ha) for wheat crop and 11.8% (4.33 and 3.82 t/ha) for maize crop with precision land leveling raised bed planting compared to traditional land leveling with flat beds planting. The agronomic efficiency (AE) of N (23.4 and 30.4 kg grain/kg N for maize and wheat) and uptake of N, P and K (103.85, 25.6 and 110.7 kg/ha for maize and 112.95, 19.49 and 112.96 kg/ha for wheat) were significantly improved under precision land leveling with raised bed planting technique compared to other practices.

Key words: Precision land leveling, productivity, profitibility, input use efficiency, permanent raised beds.

INTRODUCTION

Presently, 50% of the human population relies on nitrogen (N) fertilizer for food production. The world today uses around 83 million metric tons of N, which is about a 100-fold increase over the last 100 years (Ladha et al., 2013). About 60% of global N fertilizer is used for producing the world's three major cereals: rice, wheat, and maize. Projections estimate that 50 to 70% more cereal grain will be required by 2050 to feed 9.3 billion people (Ladha et al., 2013). Stagnating yield and declining input use efficiency in irrigated maize-wheat of the Western Indo-Gangetic Plain (WIGP) coupled with diminishing availability of water for agriculture is a major concern of food security in South Asia. Achieving sustainable food security is a major challenge considering the growing population with changing diets and a degrading resource scenario. Maize or corn (*Zea mays*) is an important food and feed crop and is one of the most versatile, high yielding food crops of the world. In South Asia with limited availability of water, and aberration in temperature on account of climate change, it is becoming increasingly important that issues concerning it be addressed. Maize lends itself to various applications and has a high yield potential and wide adaptability across regions, seasons and altitudes, making it suited in different cropping and farming systems. Its demand is increasing on account of the shift towards animal based diets and expansion of the bio-fuel industry. Raised beds were introduced to rice- wheat systems of the Indo-Gangetic Plain (IGP) in the mid 1990s, initially for wheat, inspired by the success of irrigated maize–wheat on permanent raised beds (PRB) in Mexico (Sayre and Hobbs, 2004). Since then, many advantages of growing wheat on beds have been reported, including increased yields, reduced lodging, opportunities for mechanical weeding and improved fertiliser placement, irrigation water savings, reduced waterlogging, reduced seed rate and opportunities for intercropping (Ram et al., 2005; Naresh et al., 2011a). Around the same time that PRB were being proposed, an unprecedented revolution in adoption of 'zero-till' (direct-drilled) wheat after rice was underway across the IGP (Malik et al., 2004). Majority of the

maize-wheat growers in the western Uttar Pradesh practice surface irrigation either through flood or check basin methods. The light textured soils under undulating topography leads to uneven distribution of water, which limits the availability of water and nutrients to the crop plants. Undulated crop fields when managed with flood irrigation, also lead to within field spatial variability in grain production owing to leaching of certain nutrients due to excess water at lower elevations and in-adequate availability of irrigated water at higher elevations. Naresh et al. (2011) reported that wheat and maize are planted in many parts of the world on beds and bed planting on an average saved 29% of water as compared to flat beds.

Land leveling is a precursor to good agronomic, soil and crop management practices and the levelness of the land surface has significant influence on all the farming operations. Jat et al. (2006) rated the development of laser technology for precision land leveling as second only to breeding of high yielding varieties. The soil moisture status throughout the field governed by its levelness has great influence not only on farming operations but also the yield and input use efficiency. The leveling of land for achieving higher resource use efficiency is not a new technique but the way in which it is done is not up to the mark as frequent patches of dikes and ditches stretched over a minimum workable distance are created even with best effort by conventional leveling practices. Undulated land hampers the seedbed preparation, seed placement, germination and also requires heavy draught for machines, which leads to consumption of more energy, and ultimately to more cost of production and low productivity levels. Improvement in operational efficiency, weed control efficiency, water use efficiency, nutrient use efficiency, crop productivity and economic returns (Naresh et al., 2014) and environmental benefits (Jat et al., 2006) been reported as a result of precision land leveling when compared to traditional practice of land leveling. The objective of this study was to evaluate the effect of precision land leveling and permanent raised beds planting on soil properties, input use efficiency, productivity and profitability under maize (*Zea mays*) – wheat (*Triticum aestivum*) cropping system on a sandy loam soil of western Uttar Pradesh.

MATERIALS AND METHODS

An experiment was conducted on maize-wheat rotation in three districts (Meerut, Ghaziabad and Saharanpur) 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°44 18″E) during 2009-2010 to 2011-2012 and was designed as a farmer-managed with a single replicate, repeated over many farmers. Therefore, the experimental design was Randomized Block Design in which farmer as a replicate/ block commencing with kharif in 2009. 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 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}, 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 was the predominant method of irrigation in Western UP. The plots consisted of seven crop establishment treatments and details is given here under,

T_1-Precision land leveling with wide raised Beds with recommended dose of NPK (PL WB + RNPK)
T_2 -Traditional land leveling with wide raised Beds with recommended dose of NPK (TL WB + RNPK)
T_3 -Precision land leveling with narrow raised Beds with recommended dose of NPK (PL NB + RNPK)

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T_4 -Traditional land leveling with narrow raised Beds with recommended dose of NPK (TL NB + RNPK)
T_5 - Precision land leveling with flat Beds with recommended dose of NPK (PL FB + RNPK)
T_6 - Traditional land leveling with flat Beds with recommended dose of NPK (TL FB + RNPK)
T_7Traditional land leveling with flat Beds without NPK (Control/conventional practices) (TL FB + N_0 P_0 K_0)

For laser-assisted precision land leveling, the land was first plowed at the optimum moisture level (field capacity) with a harrow/cultivator for pulverization and was leveled using a laser-equipped drag scrapper (TrimbleTM, USA) with an automatic hydraulic system attached to a 50-60 HP tractor. Before running the laser leveler, the field was surveyed at 3 m distance to record the elevation and the elevation points were averaged to know the desired elevation for leveling the field. The average elevation value was entered into the digital control box for controlling the scrapper at the desired elevation point (Naresh et al., 2011) and the tractor was run across the field till the desired elevation was achieved throughout the field. For the traditional land leveling treatment, the field was first ploughed as described above and was leveled using an iron plank attached to a tractor and was dragged across the land surface.

After ploughing with a harrow/cultivator for pulverization of the field at the optimum moisture level, an iron scraper attached to the tractor was moved on the land surface on a visual elevation level. After the cuts and fills of soil, a wooden planker attached to the tractor was moved across the field to smooth the land surface. 'HM-10' maize cultivar was seeded in the last week of June and wheat cultivar 'PBW 343' was seeded on 7^{th}, 9^{th} and 9^{th} Nov. 2009, 2010 and 2011, respectively. A seed rate of 20 kg ha^{-1} for maize and 80 kg ha^{-1} was used in treatments where wheat was seeded on beds, and 100 kg ha^{-1} was used in the rest of the treatments. The bed furrow width at top was kept at 37 cm: 30 cm having one seed rows for maize and three seed rows for wheat and the depth of the furrow was kept at 15 cm for narrow beds and furrow width at top was kept at 107 cm: 30 cm having two seed rows for maize and six seed rows for wheat and the depth of the furrow was kept at 12 cm for wide beds. The plant population was maintained equal in flat as well as raised bed planting.

Irrigation water was applied using polyvinyl chloride pipes of 15 cm diameter and the amount of water applied to each plot was measured using a water meter (Dasmesh Co., India). The quantity of water applied and the depth of irrigation were computed using the following equations:

Quantity of water applied (L) = F x t (1)

Depth of water applied (mm) = {L /A /1000} (2)

where F is flow rate (l s^{-1}), t is time (s) taken during each irrigation and A is area of the plot (m^2). Rainfall data was recorded using a rain gauge installed within the meteorological station. The total amount of water (input water) applied was computed as the sum of water received through irrigation and rainfall (I+R). Water productivity ($WP_{I}+_{R}$)(kg grains m^{-3}ofwater) was computed as follows (Humphreys et al., 2008):

WP_{I+R} =grain yield (kg ha^{-1})/[irrigation water applied (m^3)+rainfall received by the crop (m^3)]ha^{-1}. (3)

Soil samples were collected at the start of the experiment from 0 to 15 cm soil depth using an auger of 5 cm diameter. Each sample was a composite from three locations within a plot. The freshly collected soil samples were mixed thoroughly, air-dried, crushed to pass through a 2 mm sieve and stored in sealed plastic jars before analysis. Olsen P (0.5 M $NaHCO_3$ extractable) and NH_4OAc– extractable K were analyzed using the methods described by Page et al. (1982), respectively. Soil organic C was analyzed by Page et al. (1982) method. The samples for determination of soil physical properties (soil aggregates, mean weight diameter of aggregates) were collected at the start of the experiment and after the harvest of each crop. Soil aggregation and mean weight diameters of aggregates were analyzed using the wet-sieving (Yoder, 1936) method. Bulk density was measured to a depth of 20 cm at intervals of 5 cm soil depth using the core-ring method and one core per stratus of each plot was collected and the samples were oven-dried for 48 h at 105°C, weighed and bulk density calculated according to Blake and Hartge (1986). Soil moisture by gravimetric method (Jalota et al., 1998), soil strength by cone penetrometer. The bulk density were measured at the onset of the experiment and after the 3 years of study.

The plants measured for growth and yield were used for analyzing the N, P and K content in grain and straw. The grain and straw samples were dried at 70°C in a hot air oven. The dried samples were ground in a stainless steel Wiley Mill. The N content in grain and straw were determined by digesting the samples in sulfuric acid (H_2SO_4), followed by analysis of total N by Kjeldahl method (Page et al.,1982) using a Kjeltec autoanalyser. The P content (grain and straw) was determined by vanadomolybdo-phosphoric yellow colour method and the K content both in grain and straw was analysed in di-acid (HNO_3 and $HClO_4$) digests by Flame Photometeric method (Page et al., 1982). The uptake of the nutrients was calculated by multi-plying the nutrient content (%) by respective yield (kg·ha^{-1}) and was divided by 100 to get the uptake values in kg·ha^{-1} (Wu et al., 2010). The uptake in grain and straw was summed to get the total uptake of nutrient·ha^{-1}.

Nutrient use efficiency

The effectiveness of applied nutrients is to establish by this factor. The most important advantage of this index is that it quantifies total economic output from any particular factor/nutrient, relative to its utilization from all resources in the system, including native soil nutrients and nutrients from applied inputs (Dobermann et al., 2002). The following expressions are used for determining these:

Agronomic efficiency (AE_N)

AE_N = kg grain yield increase per kg N applied (often used synonym: N use efficiency:

AE_N =Δ GY_{+N} / FN (4)

Where, GY_{+N} is the grain yield in a treatment with N application kg ha^{-1}, GY_{0N} is the grain yield in a treatment without N application, and FN is the amount of fertilizer N applied, all in the kg ha^{-1}.

Recovery efficiency of applied nitrogen (RE_N)

RE_N = kg N taken up per kg N applied:

RE_N = UN_{+N} – UN_{0N} (5)

Where, UN_{+N} is the total N uptake measured in above ground biomass at physiological maturity (kg ha^{-1}) in plots that received applied N at the rate of FN (kg ha^{-1}), UN_{0N} is the total N uptake without N addition.

Physiological efficiency of applied nitrogen (PE_N)

PE_N = kg grain yield increase per kg fertilizer N taken up:

Table 1. Effect of crop establishment on bulk density, water stability of aggregates, clod breaking strength and soil organic carbon (%) etc. soil properties under maize-wheat cropping system after 3 year's of experimentation in 0 to 15 cm.

Treatment	Bulk density (Mg m^{-3})	Water stable aggregates >0.25 mm (%)	Aggregate porosity (%)	Clod breaking strength (kPa)	Soil organic carbon (%)	Field capacity (% moisture)		Permanent wilting point (% moisture)	
						0-5 cm	5-20 cm	0-5 cm	5-20 cm
T_1	1.44	82.8	43.2	204.8	0.63	31	32	13	11
T_2	1.46	79.0	40.8	332.9	0.58	29	30	11	10
T_3	1.45	81.9	42.7	235.6	0.61	30	31	12	11
T_4	1.48	72.9	40.2	367.5	0.55	29	29	11	10
T_5	1.49	80.3	41.3	289.7	0.59	29	30	12	11
T_6	1.50	66.7	39.6	418.7	0.54	28	29	11	10
T_7	1.55	59.1	36.2	423.8	0.52	28	29	11	09
CD 5%	0.09	5.3	1.74	95.3	0.53^{**}	-	-	-	-

$$PE_N = (GY_{+N} – GY_{0N}) / (UN_{+N} – UN_{0N}) \quad (6)$$

Where, GY_{+N} is the grain yield in a treatment with N application kg ha^{-1}, GY_{0N} is the grain yield in a treatment without N application, UN_{+N} are the total N uptake in a treatment with N application kg ha^{-1}, UN_{0N} is the total N uptake in a treatment without N application.

Partial factor productivity (PFP_N)

PFP_N = kg grain per kg N applied:

$$PFP_N = GY_{+N} / FN \quad (7)$$

Where, GY_{+N} is the grain yield in kg ha^{-1} and FN is the amount of fertilizer N applied in kg ha^{-1}.

RESULTS AND DISCUSSION

Soil properties

Tillage significantly affected the soil significant variations after three crop cycles the soil physical properties in bulk density, water stable aggregates, aggregate porosity, clod breaking strength, organic carbon, available N, P and K were recorded due to different treatments (Table 1). The bulk density did not varied significantly due to land leveling however, planting techniques had significant influence and it was significantly reduced under raised bed planting compared to flat sowing irrespective of the land leveling practice. This was attributed mainly due to more pore spaces created in the beds through modified land configuration by accumulations the topsoil. Bed planting provides natural opportunity to reduce compaction by confining traffic to the furrow bottoms (Govaerts et al., 2006). The soil organic carbon content in top soil (0 to 15 cm) was increased significantly due to raised bed planting compared to flat sowing planting mostly because of localized deposition of more fertile top soil on beds under altered land configuration than flat planting. Available nitrogen, phosphorus and potassium status of soil analyzed after harvest of third wheat crop showed significant variation due to different treatments (Table 2). Maximum available N, P and K content in soil was recorded under PLWB being at par with TLWB but were significantly superior to all other treatments. Further, flat planting either on precision or traditional leveling were at par with each other at similar fertility levels.

Nutrient uptake

Total (grain + straw) uptake of nutrients (N, P, K) analyzed at crop maturity varied significantly due to land leveling and crop establishment techniques. Maximum uptake of total N was recorded with PLWB which was significantly higher over all other treatments (Figure 1). Similar to nitrogen, maximum uptake of total P was also recorded in PLWB which was at par to PLNB but it was significantly higher over rest of the treatments (Figure 1). The total K uptake by the crop though at par, under precision land leveling irrespective of the planting technique (that is, PLWB, PLNB and PLFB) but significantly higher over rest of the treatments (Figure 1). The higher amount of uptake of nutrients under precision leveling and raised bed planting techniques was associated with higher bio-mass accumulation under these treatments, which led to higher amount of uptake of these nutrients. The higher nutrient uptake in

Table 2. Effect of crop establishment on available N ,available P and available K (kg/ha) under maize-wheat cropping system after 3 year's of experimentation in 0-15 cm.

Treatment	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)
T_1	261.5	13.7	247.0
T_2	250.1	12.1	241.8
T_3	259.3	13.5	245.9
T_4	249.0	11.9	240.7
T_5	258.1	13.2	244.3
T_6	243.4	11.8	240.2
T_7	139.5	8.6	232.5
CD 5%	6.7	0.40	2.15

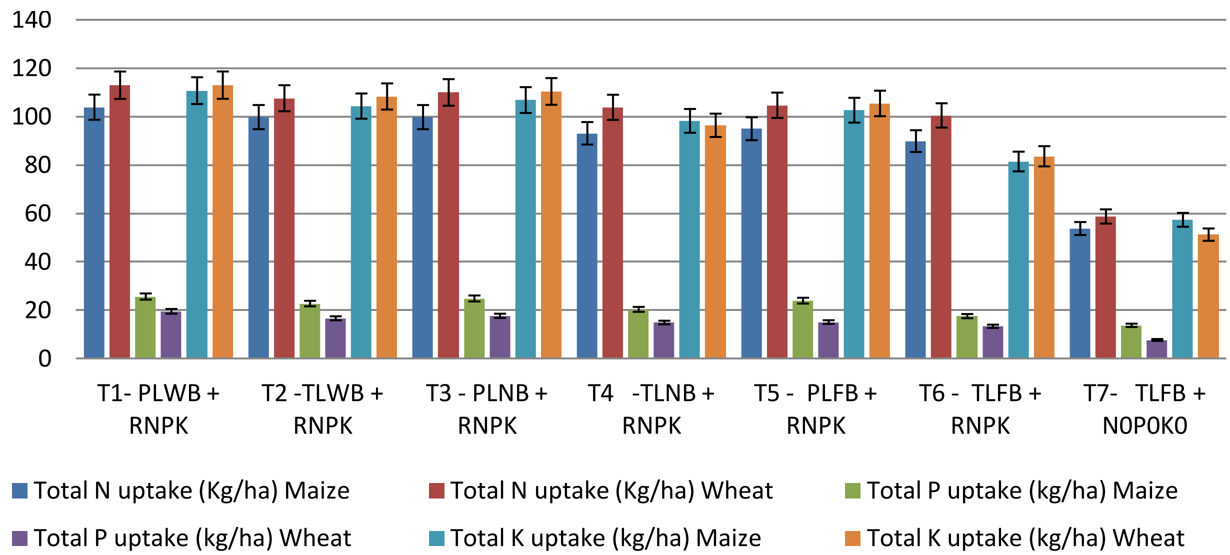

Figure 1. Effect of various tillage and establishment techniques on total N, P and K uptake in maize wheat rotation means at P = 0.05.

Precision leveling with raised beds is mainly due to less leaching loss of nutrients and availability of sufficient moisture for mineralization of native as well as applied nutrients. The higher uptake efficiency of nutrients depends on a myriod of factors including nutrient availability due to favourable soil biota under precision leveling with raised beds compared to precision leveling with flat beds. These findings are in agreement with Jat et al. (2006) and Walker et al. (2003).

Nutrient use efficiency

The agronomic as well as recovery efficiency of applied nutrients was in general higher in precision leveling permanent wide beds. Effeciencies tended to be lower in traditional leveling flat beds (TLFB) than precision leveled wide or narrow raised beds treatments. Effeciencies on raised beds consistently increased but the differences between precision leveled and traditional leveled permanent raised beds were not always significant for the maize- wheat crop cycles with the same level of nutrient application (Figure 2).

Agronomic efficiency (AE)

The agronomic efficiency (AE) of applied nutrients as unit grain production per unit of applied nutrients after deducting the soil supplying capacity was calculated for all the treatments. The AE of applied N was significantly higher under precision leveling with wide raised bed treatment compared to other treatments. The efficiency of the nutrient under PLWB + RNPK, and PLNB + RNPK was at par but significantly superior to PLFB + RNPK. The efficiency under PLFB + RNPK, TLNB + NPK and TLFB + RNPK were at par but significantly inferior to TLWP + RNPK. While traditional leveling without NPK

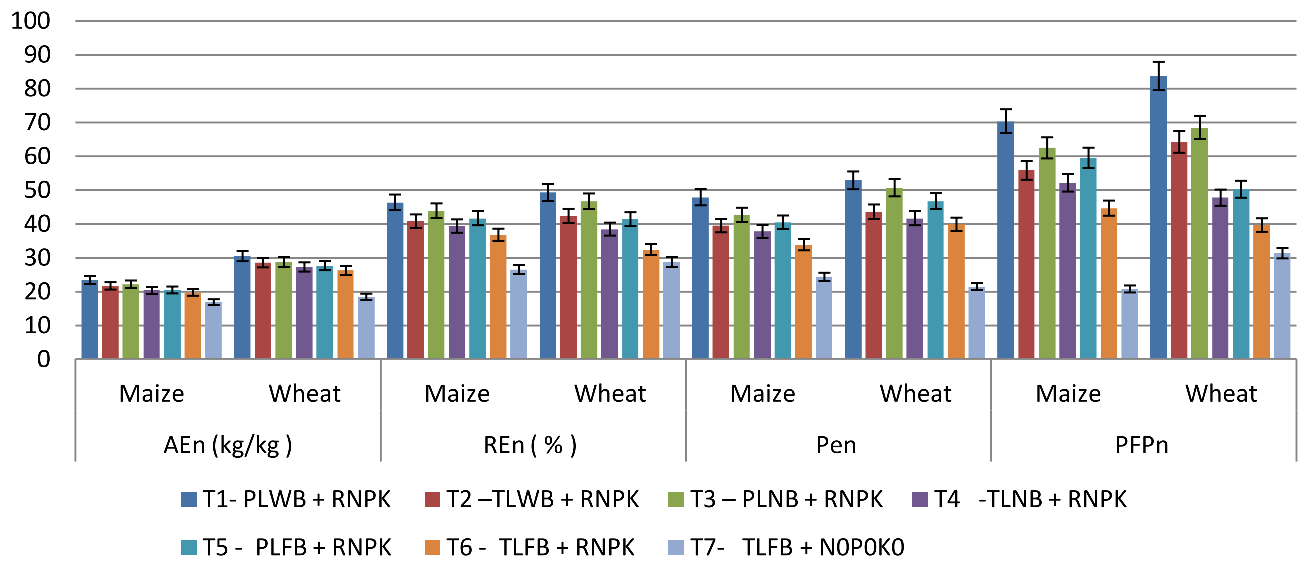

Figure 2. Estimates of N use efficiency that is, Agronomic Efficiency of N (AE_n),Recovery Efficiency of N(RE_n), Physiological Efficiency of N (Pen) and Partial Factor Productivity of N (PFP_n) in maize – wheat rotation means at P = 0.05.

(TLFB + $N_0 P_0 K_0$) had significantly lower AE than all other treatments (Figure 2).

Recovery efficiency (RE)

Data on true recovery efficiency (*REnt*) shows that in maize the values ranged from 26.4 to 46.3%, while in wheat these ranged from 28.7 to 49.7%. Maize-wheat cropping system is a new rising system for the backbone of food security in India and the values of apparent recovery efficiency of N (*REn*) of maize and wheat experiment are in Table 4. Data in Table 4 clearly show that the values of all the terms associated with N use efficiency (*NiUE*) declined in all traditional leveling plots. At N levels similar to those in Figure 2, values of all *NiUE* terms in maize were lower in compared to the wheat. On the other hand, values of all terms of *NiUE* showing that in experiment N is more efficiently utilized for wheat than maize. Thus in maize there is considerable scope to increase *NiUE*. Precision leveling irrespective of planting technique exerted significant effect on RE-N. The RE-N under PLWB+RNPK was significantly higher over all other treatments. Further, the recovery efficiency under TLLFB+RNPK also improved significantly compared to TLNB+RNPK ,TLFB+RNPK and TLFB+$N_0P_0K_0$ (Figure 2).

Water application and water productivity

The input water application includes the irrigation water applied and the rain water during the maize-wheat season of 2009 to 2010 to 2011 to 2012.The water application in maize-wheat system was remarkably lower with permanent wide and narrow beds compared to other practices (Tables 3 and 4). The higher irrigation water application in maize- wheat system under traditional leveling treatments as compared to precision leveled plots. The precision leveled plots savings in water use in raised beds with recommended dose of NPK were 11.5 to 20.5% in maize and 14.1 to 26.7% in wheat as compared to traditional leveled flat beds with recommended dose of fertilizer treatment (T_6). Water productivity under permanent beds was higher as compared to other tillage and crop establishment techniques and lowest system water productivity was recorded with traditional leveled flat beds. Bhushan et al. (2007) revealed that the saving in irrigation water with raised bed planting technique was more under traditional leveling as in this technique water moves in furrows only. Laser assisted precision land leveling can reduce evaporation and percolation losses from wheat by enabling faster irrigation times and by eliminating depressions and therefore ponding of water in depressions.

Grain yields

The crop yield data from 2009-2012 (Table 1) showed that the higher grain yields of maize occurred in precision land leveling permanent wide beds with recommended dose of NPK. Yields on raised beds consistently increased from 1 to 3 year in laser leveling with recommended NPK, but the differences between laser leveling permanent wide raised beds and permanent narrow raised beds were not always significant for the three maize- wheat crop cycles.

Table 3. Water productivity and profitability of maize and wheat rotation under various tillage and establishment techniques.

Crop establishment	Water productivity (kg yield m^{-3} water)						Net profit (Rs ha^{-1})					
	Maize			Wheat			Maize			Wheat		
	2009	2010	2011	2009-2010	2010-2011	2011-2012	2009	2010	2011	2009-2010	2010-2011	2011-2012
T_1- PLWB + RNPK	1.31	1.42	1.53	1.75	1.82	2.06	23580	24680	25375	23585	23875	24560
T_2 -TLWB + RNPK	1.16	1.17	1.17	1.54	1.52	1.50	22320	22495	23310	21525	21650	21985
T_3 - PLNB + RNPK	1.24	1.33	1.39	1.57	1.69	1.78	22765	23570	24125	22350	22790	23275
T_4 -TLNB + RNPK	1.10	1.11	1.12	1.41	1.40	1.36	21550	22200	23050	21300	21435	21525
T_5 - PLFB + RNPK	1.10	1.15	1.22	1.40	1.45	1.52	20910	21375	21890	21750	21975	22430
T_6 - TLFB + RNPK	1.01	1.00	1.01	1.25	1.19	1.19	19500	19760	20300	20050	19610	19250
T_{7-} TLFB + $N_0P_0K_0$	0.60	0.52	0.49	0.76	0.70	0.59	12650	11275	10725	11950	10990	09725

Table 4. Maize and wheat water application (mm ha^{-1}) and crop yield (t ha^{-1}) in laser-leveled and traditionally leveled field under different tillage and crop establishment methods.

Crop establishment	Maize						Wheat					
	2009		2010		2011		2009 - 2010		2010 - 2011		2011 – 2012	
	Water	Yield	Water	Yield	Water	Yield	Water	Yield	Water	Yield	Water	Yield
T_1- PLWB + RNPK	325	4.25	310	4.40	300	4.60	295	5.15	285	5.20	260	5.35
T_2 -TLWB + RNPK	345	4.00	350	4.05	360	4.20	305	4.70	310	4.72	320	4.80
T_3 - PLNB + RNPK	330	4.10	320	4.25	312	4.35	315	4.95	295	5.00	290	5.15
T_4 -TLNB + RNPK	355	3.90	360	4.00	370	4.15	330	4.65	335	4.68	345	4.70
T_5 - PLFB + RNPK	360	3.95	350	4.02	345	4.20	340	4.75	330	4.77	320	4.85
T_6 - TLFB + RNPK	370	3.75	375	3.80	385	3.90	355	4.45	370	4.42	375	4.45
T_{7-} TLFB + $N_0P_0K_0$	385	2.30	390	2.05	397	1.95	360	2.75	365	2.55	380	2.25
C D at 5 %	-	0.21	-	0.29	-	0.37	-	0.24	-	0.23	-	0.31

Precision leveling with residue retain increase the yield by 8.5 to 10.9% in maize and 10.1 to 13.1% in wheat as compared to conventional seeding. This is an extremely important finding in relation to practical management of such systems by farmers. Data pertaining to crop yield parameters of wheat (Table 4) showed significant variation due to land leveling and planting techniques during the study years. The yield level, in general, under all the treatments was little higher during year 3 compared to year 1 and 2. This was attributed mainly due to more sunshine hours cross the season in year 3 compared to year 1 and 2. Also, the minimum temperature during flowering season was higher during year 1 and 2 compared to year 3 which limits the reproductive period and responsible for lower yields of wheat. Grain yield of wheat varied significantly due to laser leveling permanent wide raised beds with recommended dose of NPK (PLWB +RNPK) techniques and significantly higher yield levels of 5.15, 5.20 and 5.35 t·ha^{-1} were recorded under (PLWB+ RNPK) during year 1, 2 and 3, respectively compared to other treatments. The increase in grain yield with (PLWB+ RNPK) was 8.3, 8.7 and 9.3% during year 1, 2 and 3, respectively whereas the corresponding increase under flat bed planting was recorded at 6.3, 7.3 and 8.2%. The yield under permanent wide raised beds traditional land leveling with recommended dose of NPK (TLWB+RNPK) and and laser leveling permanent narrow raised beds with recommended dose of NPK (PLNB+RNPK) did not varied significantly during the years because productive tillers, length of spike and number of grains/spike are almost same. Further, with the same level of land leveling and different levels of planting techniques, the wheat yield varied remarkably. Raised bed showed 5.9, 6.4 and 7.6% yield advantage over flat bed planting under precision leveling during year 1, 2 and 3, respectively whereas, the corresponding increase in yield under traditional leveling was recorded at 4.7, 5.9 and 6.3%. It showed that the raised bed planting technique is more advantageous under precisely leveled fields. Significantly higher yield of maize-wheat was recorded with precision land leveling as it takes care of maintaining near homogeneity by way of cut and fill and also tillage (Hassan et al., 2005; Borrell and Garside 2004). These findings are in agreement with Gupta and Sayre (2007); Rajput and Patel (2004) and Naresh et al. (2011a, b) who summarized the finding of multilocation trails across IGP and reported higher yield of wheat with raised beds compared to flat sowing.

Profitability

The net income through maize was higher with precision leveling permanent raised beds with recommended dose of NPK followed by PLNB+RNPK>TLWB+RNPK>TLNB+RNPK and PLFB+RNPK and the lowest being recorded with TLFB+$N_0P_0K_0$ (Table 3). The lower net income with the conventional tillage was due to the cost on preparing the field. Profitability of wheat was remarkably higher with precision leveling permanent wide raised beds practices due to higher productivity and less cost of production compared to conventional tillage practices (T_7) treatment. 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 maize-wheat rotation in the western U. P. Precision land leveling and permanent beds planting with recommended dose of NPK have a tremendous potential for improving the use efficiency of natural as well as externally applied resources. Many new opportunities based on precision land leveling and permanent beds planting with recommended dose of NPK have appeared to give stimulus to the productivity through a more sustainable pace of natural resource use in maize-wheat based cropping system. Taken together, these practices can rise productivity, cut costs, save water and soils, reduce use of external inputs, foster greater agro-ecosystem diversity and generate employment. Lignified residual straw and roots added more organic matter and nutrients into the soils under permanent raised beds, resulting in increased nutrient uptake by the crops. Crop yields on beds with precision land leveling rose by about 11.8% for maize and 13.5% for wheat over 3-year cycle compared with conventional tillage on the flat beds.

FUTURE THRUST OF RESEARCH

(i) Conservation tillage practices like precision land leveling and permanent bed planting utilizes more judiciously the plant available water than the conventional tillage when the other factors are similar.

(ii) Precision-Conservation Agriculture (PCA) based crop management solutions seem to be promising options to sustain the maize- wheat systems of South Asia on a long-term basis.

Conflict of Interests

The author(s) have not declared any conflict of interests.

Abbreviations: PLWB, Precision land leveling with wide raised Beds (Tops of the beds 107 cm and Furrow width 30 cm and 12 cm depth); **TLWB,** Traditional land leveling with wide raised Beds (Tops of the beds 107 cm and Furrow width 30 and 12 cm depth); **PLNB,** Precision land leveling with narrow raised Beds (Tops of the beds 37 cm and Furrow width 30 and 15 cm depth); **RNPK,** recommended dose of NPK (120:60:40 kg/ha); **PLFB,** Precision land leveling with flat Beds.

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Full Length Research Paper

Studies on bio-ecology and voracity of leaf roller (*Diaphania indica* Saunders, Lepidoptera: Pyralidae) on pointed gourd (*Trichosanthes dioica* Roxb.)

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Field and laboratory studies have been carried out on the bio-ecology of *Diaphania indica* Saund. Correlation coefficient with all seven weather factors considered in the present study indicated that there existed significantly high negative correlation with five factors *viz.* temperature (°C), relative humidity (%), rainfall and soil temperature (°C) but bright sun shine hours that did bear significant weak positive correlation. Predictive models were developed from pooled data of two years' (2008 and 2009) observations on population *vis-à-vis* weather factor; one with irrespective of whether they had significant effect on population performance or no, and another with the significant ones. Biology of leaf roller larvae studied under laboratory condition done at temperature varying between 30 and 35°C showed that the two temperature regimes had little effects on duration of different developmental stages. The total developmental period was 16 to 22 days (20.3±1.76 days) in 30°C and 17 to 22 days (19.5±1.43 days) in 35°C. Consumption and efficiency of conversion of ingested food to biomass was worked out through established procedures. Consumption increased progressively through five larval instars from initially 3.74 to finally 81.25 and its conversion of biomass from 3.96 to 140.02 as indicated by indices.

Key words: Bio-ecology, voracity, correlation, predictive model, biology.

INTRODUCTION

Diaphania indica (Saunders) (Lepidoptera: Pyralidae) known as pumpkin caterpillar, is one of the major pests of most cucurbits all over the world (Morgan and Midmore, 2002; Arcaya et al., 2004). The pest attacked on different cucurbitaceous crop and has been reported from several states in India (Ke et al., 1988; Peter and David, 1990; Ravi et al., 1997, 1998; Ambarish and Maitreyi, 1998; Radhakrishnan and Natarajan, 2009). It has also been reported to attack pointed gourd (Chintha et al., 2002; Jhala et al., 2005). On hatching, larvae feed on leaves where they cluster and fold and weave the leaves together. They can also feed on and puncture the skin of young fruit, especially the fruits that touch leaves (Patel and Kulkarny, 1956; Namvar and Alipanah, 2002; Sobrinho et al., 2003; Arcaya et al., 2004; Jhala et al., 2005). The damage by larvae has been reported to be restricted to leaves of pointed gourd which ranged from 25 to 30% (Jhala et al., 2005) which, in bitter gourd was 3

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to 14% only (Singh and Naik, 2006). The close relationship between population dynamics of any pest and weather can form the basis of pest forewarning system (Chinniah et al., 2007). The said relationship has been worked out in present study in order to understand bio-meteorological interactions. The seasonal incidence of the pest had been recorded and the impact of some of the weather factors *viz.* temperature maximum and minimum, RH% morning and evening, total rainfall, soil temperature and bright sun shine hours had been analysed. Alongside this biology and food consumption of the pest had also been studied. This will help in preparation of management design of the pest.

MATERIALS AND METHODS

Pointed gourd crop *cv.* Dhapa was raised in field conditions at Central Research Farm of the Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, West Bengal, India. The population dynamics of leaf roller was monitored in three consecutive years from 2008 to 2010. Experiments were replicated three times with each plot having the size of 6 m^2 (3 m × 2 m) with a plant spacing 100 cm × 135 cm accommodating six females and one male root cuttings in pits. FYM at 150 q/ha and N, P and K were applied at 90, 80 and 70 kg/ha respectively. Mature root cuttings were planted in three seasons during November to August in 2007-2008, 2008-2009 and 2009-2010. The harvesting of fruits was done at regular interval starting from March-April to August which took 260 to 270 days for attaining maturity. Observations on the pest activity were recorded after beginning of the infestation at weekly intervals till the crop attained maturity. The numbers of leaf roller larvae were counted directly from two randomly selected tagged plants in each of the replications and average population per leaf was estimated. The weekly meteorological data on maximum temperature; minimum temperature, morning relative humidity, evening relative humidity, rainfall, soil temperature and bright sun shine hours were obtained from the Department of Agril. Meteorology and Physics, Bidhan Chandra Krishi Viswavidyalaya.

The culture of leaf roller was maintained on pointed gourd leaves in the laboratory of Plant Health Clinic, Directorate of Research, Bidhan Chandra Krishi Viswavidyalaya. Freshly handpicked mature larvae were brought to the laboratory and then reared in a plastic jar with its mouth tightly covered with a fine muslin cloth tied with rubber band. The larvae were fed with fresh, non-infested pointed gourd leaves. Pupae were collected and transferred to another jar with a fresh tender twig held in a small conical flask containing water and plugged with cotton. On emergence a pair of the female and male was confined in another jar for mating. The jar was provided with diluted honey-glucose solution-soaked cotton. Females laid eggs not only on tender twig either singly or in batches but also on jar walls and muslin cloth. The male and the female were distinguished basing on the presence of bushier anal tuft of brown hairs that can be found on females only. Ten newly hatched larvae were transferred with the help of a soft camel hair brush to the petridishes (5 × 1 cm) and were provided with tender pointed gourd leaves. The bottom of each petridish was lined with a slightly moistened filter paper to prevent desiccation. The leaves were replaced every day with fresh ones and faecal matters were removed. The larval development was observed daily. After completion of the larval development at 5^{th} instar, those went for pupation within leaves folded by spinning web which were kept seperately to record pupal duration.

For voracity study, freshly laid eggs were collected and brought to the laboratory to study of efficiency of conversion of ingested leaf of pointed gourd. The larvae after hatching were reared on fresh leaves of pointed gourd collected from the field. The first instar stage was raised in a common culture within plastic cages. The larvae were segregated just after the first larval moult and about 10 larvae were separately reared in Petri dishes, that were provided with known weight of leaves of almost same size and equal quantity of leaves were taken as aliquot to determine the dry matter content. The larval weights were taken just after moulting. Five larvae of each instar from first to the prepupal stage were weighed and dried to determine the dry weight of such larvae. The total quantity of food consumed by the larvae of each instar was determined by subtracting the weight of left over after feeding from the weight of leaves supplied. The utilization of food was worked out following Waldbauer (1964). The drying of food and the faecal matters was done in a hot air oven at 100°C till the weight was constant.

RESULTS AND DISCUSSION

Biology of leaf roller *D. indica* (Saunders)

The eggs were laid singly or in small clusters on the lower surface of leaves, leaf buds and young stems. Eggs were very small, round in shape and yellow in color. Freshly laid eggs measured from 0.73 mm. to 0.95 mm. in length and 0.30 mm. to 0.45 mm. in width (Table 2). Incubation period was 3.7±0.48 days at 30°C and 3.2±0.42 days at 35°C (Table 1). On hatching, they passed through five larval instars to become adult. The length and width of different instars had been presented in Table 2. The larval period was 9.5±1.43 days at 30°C and 8.4±1.17 days at 35°C. Time requirement to complete different instars had been presented in Table 1. Pupation took place within a white silky cocoon, remaining attached to leaves which were rolled by the caterpillars prior to pupation. The length and width varied from 13.00 to 14.20 mm and 2.54 to 3.03 mm respectively (Table 2). The pre-pupal and pupal periods were 1.9±0.56 and 8.9±1.19 days at 30°C and 2.2±0.78 and 9.1±1.44 days at 35°C respectively (Table 1). The total developmental period from egg to adult emergence was averaged to 20.3±1.76 days at 30°C and 19.5±1.43 days at 35°C (Table 1). The length of the adult varied from 13.80 to 14.80 mm and width from 3.00 to 3.60 mm (Table 2). The longevity of adult moth was 9.5±1.17 days at 30°C and 8.7±1.16 days at 35°C (Table 1). The present finding was more or less in conformity with that of Ganehiarachchi (1997) who reported incubation, larval and pupal period to be 3-5 days, 8-10 days, 7-9 days at room temperature respectively on snake gourd and Korgaonkar et al. (2004) who reported it to last for 4.75 days, 11.9 days and 9.4 days respectively on little gourd. The duration of mean developmental period from oviposition to adult emergence and the longevity of adults were ranged from 23 to 33 days with an average of 27.35 and 8.45 to 9 days respectively. Shin et al. (2002) reported that the adult period of the pest on melon was 21 days and on cucumber was 15.5 days. However, no reference could be found out on such study on pointed gourd.

Table 1. Biology of leaf roller, *Diaphania indica* (Saunders) on pointed gourd.

Stage	Duration in days (X±SD) (30°C)	Duration in days (X±SD) (35°C)
Egg period	3.7±0.48 (3-4)	3.2±0.42 (3-4)
Larval longivity	9.5±1.43 (7-11)	8.4±1.17 (6-10)
1st instar	2.2±0.63 (1-3)	1.3±0.48 (1-2)
2nd instar	2.6±0.52 (2-3)	1.7±0.48 (1-2)
3rd instar	1.8±0.42 (1-2)	1.4±0.52 (1-2)
4th instar	1.6±0.51 (1-2)	1.6±0.51 (1-2)
5th instar	1.3±0.48 (1-2)	2.4±0.69 (2-4)
Pre pupal period	1.9±0.56 (1-3)	2.2±0.78 (1-3)
Pupal period	8.9±1.19 (7-11)	9.1±1.44 (7-12)
Total developmental period	20.3±1.76 (16-22)	19.5±1.43 (17-22)
Adult longivity	9.5±1.17 (8-11)	8.7±1.16 (7-11)

Table 2. Measurement of different life stages of *D. indica* (Saunders).

Life stage	Length (mm) Mean±S.D.	Width (mm) Mean±S.D.
Egg	0.83±0.08 (0.73 - 0.95)	0.38±0.05 (0.30 - 0.45)
Larval Instar I	3.90±0.24 (3.80 - 4.30)	0.50±0.07 (0.40 - 0.56)
Larval Instar II	6.26±0.61 (5.00 - 7.10)	0.86±0.10 (0.64 - 1.02)
Larval Instar III	9.71±0.89 (8.40 - 11.20)	1.39±0.15 (1.22 - 1.66)
Larval Instar IV	14.67±0.52 (13.80 - 15.40)	2.15±0.20 (2.00 - 2.50)
Larval Instar V	18.04±0.46 (17.20 - 18.50)	3.21±0.13 (3.01 - 3.36)
Pupae	13.63±0.39 (13.00 - 14.20)	2.83±0.15 (2.54 - 3.03)
Adult	14.26±0.35 (13.80 - 14.80)	3.17±0.24 (3.00 - 3.60)

Seasonal history and relationship between leaf roller population and different weather parameters

Leaf roller, *D. indica*, could be recorded throughout the crop season during all three years of 2008, 2009 and 2010. Population was very low during each of the years of study being 0.01 to 0.12 per leaf in 2008, 0.01 to 0.17 in 2009 and 2010. It required 110 days in 2008, 65 days in 2009 and 112 days in 2010 for first appearance of the pest. Population was relatively high in early phase, that is, during March 2008, during January 2009 and again during March 2010. It fluctuated sometimes considerably as during 2009 being not so copious during other two years. Variation in the infestation levels of the insect could be noted which could have been influenced by the weather factors. In the present study the impact of weather factors on the population build up and development of predictive models thereof have been tried.

Relationship between leaf roller population and temperature

It was observed that during 2008, leaf roller population was active throughout the growing season of the crop. Data was recorded from the beginning of March. The population reached its peak (0.12 larvae/leaf) during second week of March when the weekly average maximum temperature was around 30°C (Figure 1). During April and June, leaf roller population was a bit lower. During 2009, leaf roller appeared in the first week of January and peak population was observed during third week of January when the weekly average maximum temperature was around 27°C (Figure 2). Hence, it may be inferred that when weekly average maximum temperature was around 27 to 30°C, the peak leaf roller population could be observed.

With respect to relationship of number of leaf roller population per leaf with minimum temperature the highest number of leaf roller was found during beginning of March, 2008 and third week of January, 2009 when the weekly average minimum temperature attained the value of 18 and 14°C, respectively (Figures 1 and 2). In respect to soil temperature, it was around 20 and 17°C (Figures 1 and 2). Hence, it may be deducted that when weekly average minimum temperature and soil temperature were around 14-18 and 17-20°C, the peak leaf roller population could be observed. The two years' data indicated that leaf roller population was negatively and significantly

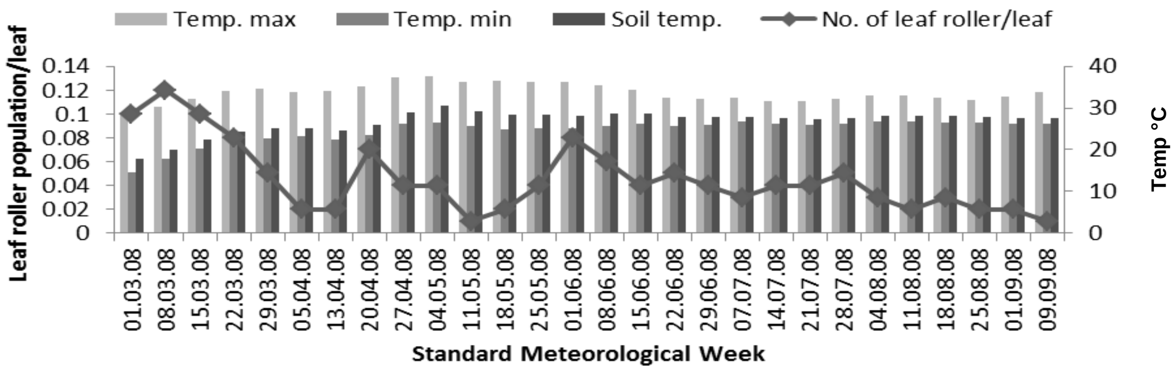

Figure 1. Influence of temperature on leaf roller population during 2008.

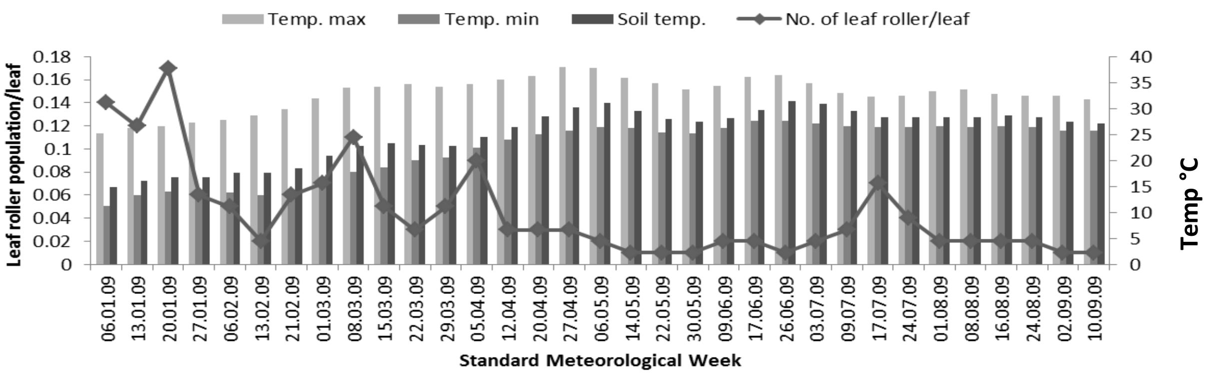

Figure 2. Influence of temperature on leaf roller population during 2009.

correlated with maximum temperature (r = -0.523), minimum temperature (r = -0.774) and soil temperature (r = -0.763). Hence, for formulation of predictive equation minimum weather and soil temperature were considered due to their strong association.

Relationship between leaf roller population and relative humidity

During 2008, peak population (0.12 larvae/leaf) reached during beginning of March when the weekly average morning relative humidity was around 93% (Figure 3). During April and June, leaf roller population was below the highest population and the weekly average morning relative humidity was around 87 to 91%. During 2009, the pest appeared in the first week of January and peak population was observed during that time when the weekly average morning relative humidity was around 95%. Hence, it may be deducted that when weekly average morning relative humidity was around 93 to 95% (Figure 4), the peak leaf folder population could be observed.

The weekly average evening relative humidity attained the value of 50% during beginning of March, 2008 (Figure 3) and 58% during first week of January, 2009 (Figure 4) which coincided with maximum leaf roller population. Hence, it may be deducted that when weekly average evening relative humidity was around 50-58% the peak leaf roller population could be observed.

The two years' data indicated that leaf roller population was negative but non-significantly correlated with morning relative humidity (r = -0.029) and negative but significantly correlated with evening relative humidity (r = -0.535). Hence, for formulation of predictive equation evening relative humidity was considered.

Relationship between leaf roller population and rainfall

It was observed that during 2008, peak population (0.12 larvae/leaf) reached during beginning of March when there was no or trace rainfall (Figure 5). During April and June, leaf roller population was below the highest population and the weekly rainfall was around 4 to 29 mm. During 2009, leaf roller appeared in the first week of January and peak population was observed during that time when the weekly rainfall was around 0 mm (Figure 6). Hence, it may be deducted that population of leaf

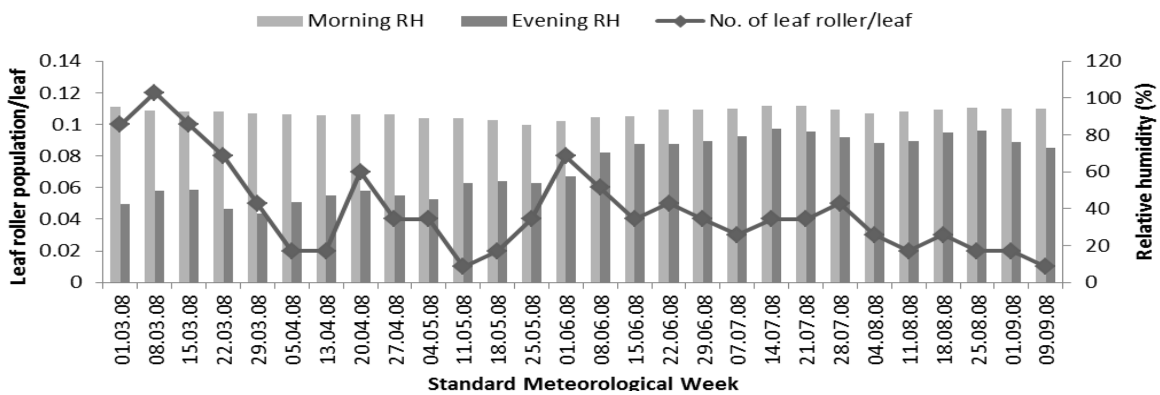

Figure 3. Influence of relative humidity on leaf roller population during 2008.

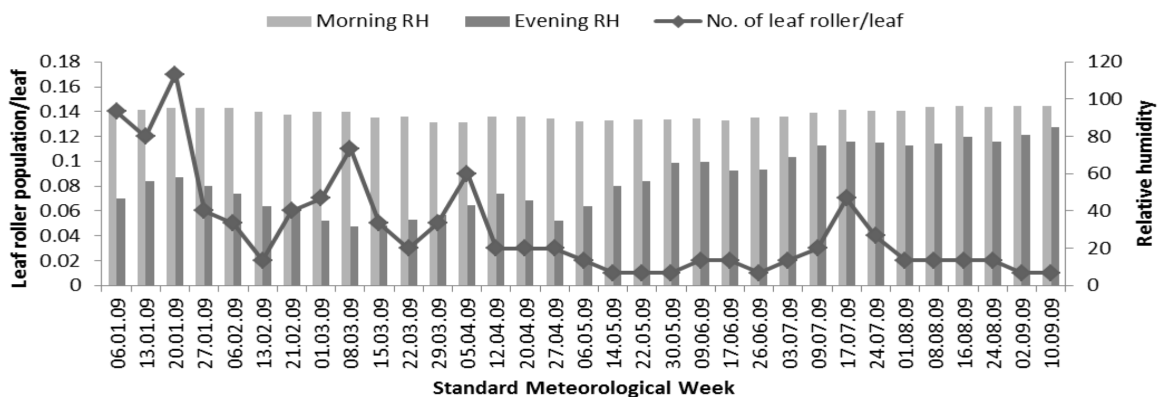

Figure 4. Influence of relative humidity on leaf roller population during 2009.

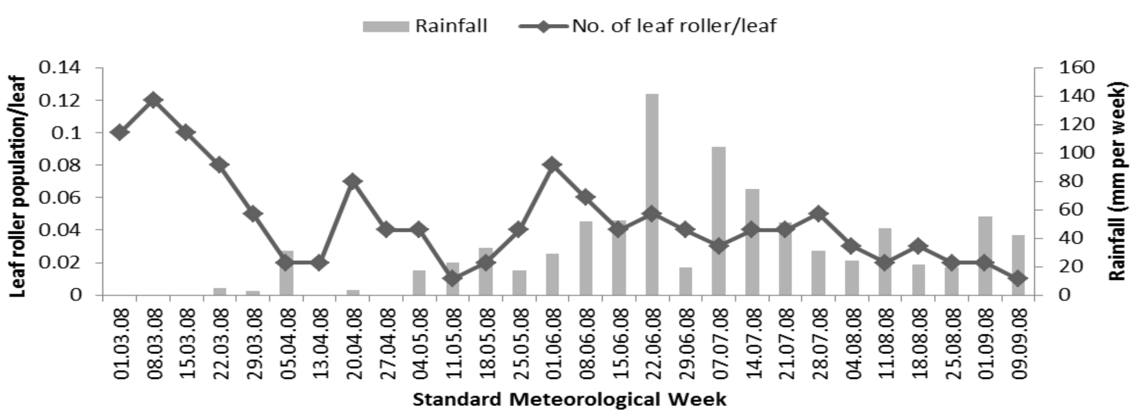

Figure 5. Influence of rainfall on leaf roller population during 2008.

roller better performed under nil or trace rainfall conditions.

The two years' data set indicated that leaf roller population was negatively and significantly correlated with rainfall (r = -0.524). Hence, for formulation of predictive equation this weather parameter was considered.

Relationship between leaf roller population and bright sun shine hour

Population peak during March 2008 and January 2009 occurred when the weekly average bright sun shine was 8 and 6 h respectively (Figures 7 and 8). The small high populations during the remaining period of crop season

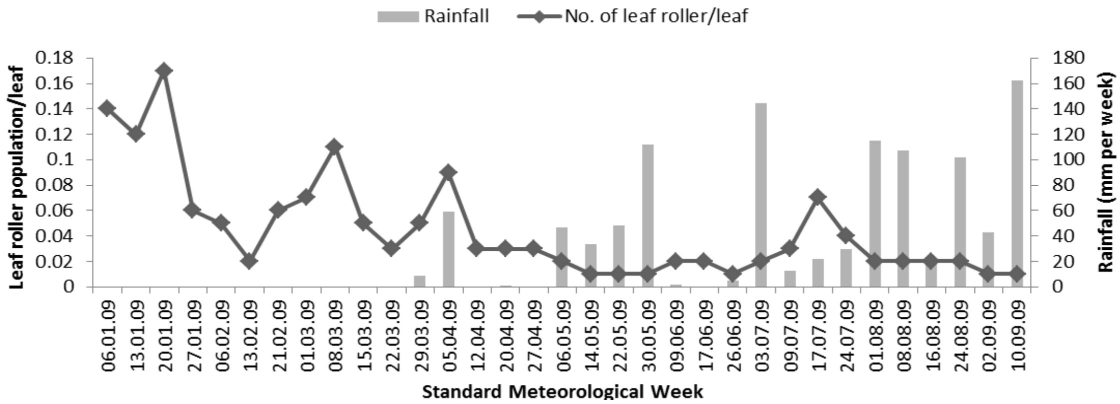

Figure 6. Influence of rainfall on leaf roller population during 2009.

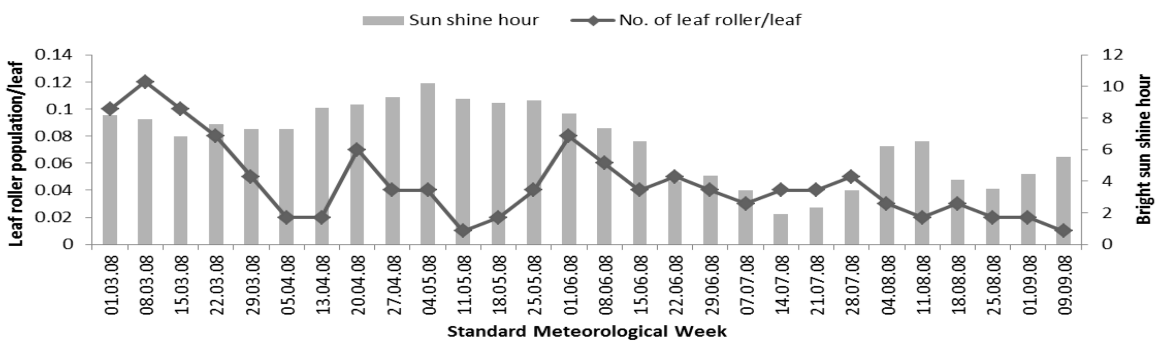

Figure 7. Influence of sun shine hour on leaf roller population during 2008.

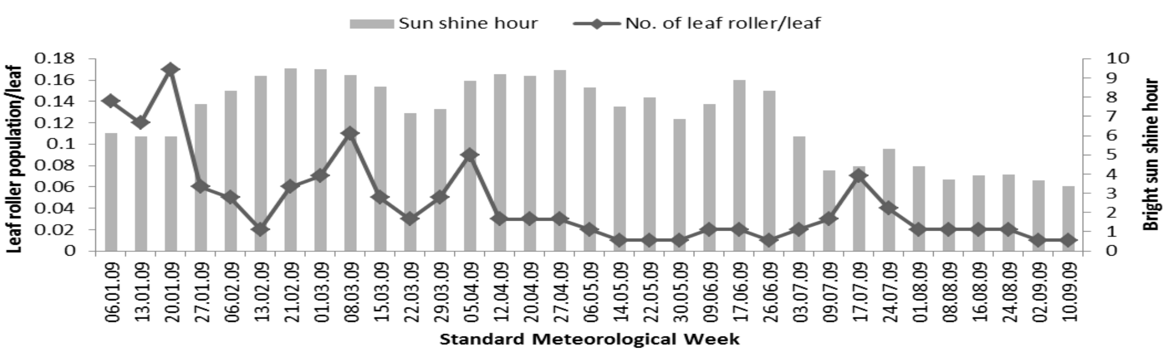

Figure 8. Influence of sun shine hour on leaf roller population during 2009.

did never shoot up to the level of peak incidence records and during this period the sun shine hour was more than eight. Hence, it may be deducted that when weekly average bright sun shine hour was around 6 to 8 h, the peak leaf roller population could be observed.

The two years' data set indicated that leaf roller population was negatively and significantly correlated with bright sun shine hour (r = -0.396). Hence, for formulation of predictive equation this weather parameter was considered.

Development of predictive equation to forewarn leaf roller population

The population dynamics of leaf roller influenced by different weather parameters, as discussed previously, were used to develop forewarning model. The data of first two years (that is, 2008 and 2009) were used for developing predictive equation and the last year's data were used for validation purpose.

As per the discussion on leaf roller-weather relationship

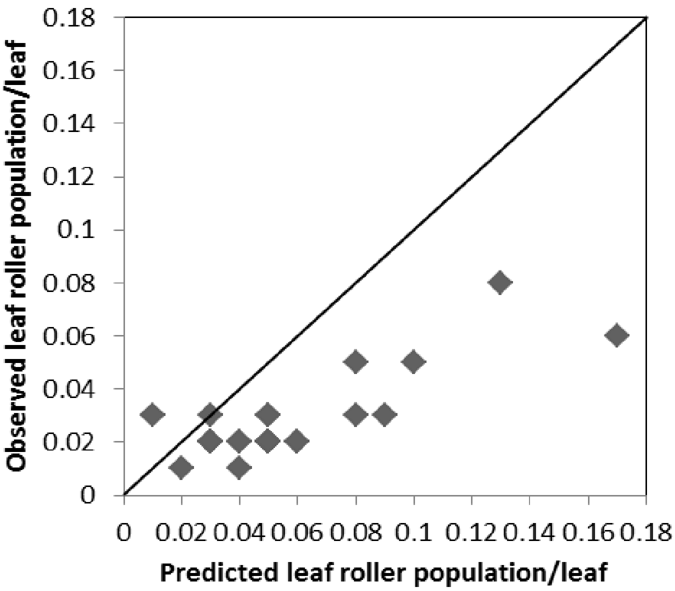

Figure 9. Comparison between observed and predicted leaf roller population per leaf.

done in the previous section minimum temperature, evening relative humidity, rainfall, soil temperature and bright sun shine hour were considered for development of predictive equation. The equation developed in this study was as follow:

$Y = 0.103 - 0.012X_1 + 0.001X_2 + 0.000X_3 + 0.005X_4 + 0.004X_5$ $R^2 = 0.632$

Where, Y–Mean no. of leaf roller/leaf; X_1, Minimum temperature; X_2, Evening relative humidity; X_3, Rainfall; X_4, soil temperature; X_5, Bright sun shine hour. Using all the weather parameters, the equation stands as:

$Y = 0.489 + 0.005X_1 - 0.024X_2 - 0.005X_3 + 0.002X_4 + 0.000X_5 - 0.011X_6 - 0.003X_7$ $R^2 = 0.664$

Where, Y, mean no. of leaf roller/leaf; X_1, maximum temperature; X_2, minimum temperature; X_3, morning relative humidity; X_4, evening relative humidity; X_5, Rainfall; X_6, soil temperatures; X_7, bright sun shine hour.

Comparison between observed and predicted leaf folder population

The predicted and observed leaf roller population per leaf was done through 1:1 line. It was observed that most of the data points were along with 1:1 line and from the Figure 9 it is apparent that the predicted leaf roller population was consistently much lower than that observed. Thus the prediction is much safe and may allow time for arranging management strategies. The root mean square error value was 0.037, indicating the developed equation can be used safely to predict the leaf roller population on pointed gourd. Thus the leaf roller population is very much closely related with minimum temperature, evening relative humidity, rainfall, soil temperature and bright sun shine hour. The predictive equation thus formed through the present investigation can form the basis of forewarning system towards better pest management of pointed gourd.

From the aforesaid discussion it can be inferred that all abiotic factors other than morning relative humidity influenced significantly the incidence pattern leaf roller population. Multiple correlation analysis with climatological data showed that pest population had significant negative correlation with maximum and minimum temperature. The incidence was high, during the first fortnight of March in 2008, third week of January in 2009 and second week of March in 2010 when the weekly average temperatures ranged from 17.7 to 30.3°C. However, this finding contradicted the observation of Das (2001) who reported that maximum and minimum temperature had significant positive relation with the pest population. The present study further revealed that relative humidity and rainfall had significant negative relation with population buildup of the pest. These findings are also in disagreement with the findings of Das (2001) who reported that relative humidity and rainfall had no significant effect on leaf roller population on pointed gourd. Further to this, present study revealed that the soil temperature was negatively and bright sun shine hour positively correlated with population build up. Through this present piece of study the predictive models to forewarn the incidence of *D. indica* (Saunders) on pointed gourd had been developed. No earlier reference on such model development on the pest of this crop had been found.

Efficiency of conversion of ingested leaf of pointed gourd by *Diaphania indica* (Saunders)

The larvae just after hatching from egg were too small. The weight gained by the larvae through development was spectacular which ultimately attained an average of 120 mg when it became full grown after four larval moults. However, at the end of the 5th instar, that is, during prepupal stage, the larvae stopped feeding and their body shrinked. They also defecated. This resulted in decrease in body weight. During feeding each larvae fed on an average 468.9 mg. of green leaf. Therefore, it may be assumed that a larva, to complete its development, may consume even less than 0.5 gm green matter of the plant.

The conversion of plant biomass into insect biomass is indicated by the rate of consumption and utilization of pointed gourd leaf as food by this insect. The various indices presented in Table 3 revealed that during the development, increase in mean dry weight of larvae at

Table 3. Consumption and utilization of pointed gourd leaf by different larval instar of *D. indica* (Saunders).

Characteristics	Larval instar				
	I	II	III	IV	V
Larval period (days)	2.2±0.63	2.6±0.52	1.8±0.42	1.6±0.51	1.3±0.48
Food consumed (dry matter mg)	5.35	33.1	35.8	69.7	73.8
Faecal matter (dry weight mg)	0.3	9.4	13	28	31
Consumption index (C.I.)	2.21	2.01	1.14	0.84	0.47
Average digestibility (A.D.)	94.39	71.63	63.74	59.83	58.02
Efficiency of conversion of ingesta	3.74	15.09	30.68	57.39	81.25
Efficiency of conversion of digesta	3.96	21.07	48.14	95.92	140.02

various instar tended to increase with the advancement of larval instars. This has also been indicated by various workers with different insects (Chlodny, 1967; Bhat and Bhattacharya, 1978). Consumption index (C.I.) of *D. indica* (Saunders) decreased gradually as the insect grew from first to fifth instar. This indicated that the rate of consumption of food though increased with the advancement of growth stages, it was not reflected aptly in the gain in weight of the larvae. This happened due to decrease of the average digestibility (A.D.) of the larvae in the all the succeeding stages. Efficiency of conversion of ingested food (E.C.I.) and efficiency of conversion of digested food (E.C.D.) into body matter in leaf roller larvae increased gradually at galloping rate all through from first to fifth instar. This indicated gradual increase in physiological activity of the larvae. Mukherjee and LeRoux (1969) in *Podius maculiventris*; Bhat and Bhattacharya (1978) for *Spodoptera litura* and Pal and Ghosh (1986) for *Acherontia* styx, Chlodny (1967) in *Leptinotarsa decemlineata* also reported identical phenomenon.

It, therefore, appears that not only the rate of feeding And relative utilization of ingested food varied from one species to another, the utilization of food consumed related to the growth of insect was also specific. The efficiency of utilization of food also varied between the instars of the same species.

Conflict of Interests

The authors have not declared any conflict of interests.

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