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*Full Length Research Paper*

# Phenotypic plasticity in soybean (*Glycine max* (Merrill)) genotypes with contrasting growth characteristics subjected to planting density stress at different developmental stages

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**A study was conducted to determine phenotype plasticity responses in soybean genotypes subjected to plant density stress at different phenological stages. Plants planted at supra optimal population density (500,000 plants per hectare) were thinned to 400,000 population density at 4 developmental stages: V1 (Full expansion of first trifoliate leaves), V3 (Four nodes with 3 unfolded trifoliates), V6 (Seven nodes, with 6 unfolded-unifoliate) and R2 (Full bloom with an open flower at the top). The study was conducted in Chilanga, Zambia. Three varieties: Dina, an indeterminate variety and Magoye and Sc Safari determinate varieties were used. A randomized complete block design arranged as a factorial design with two treatments: varieties and thinning stage and four replications were used. Variety had significant effects on days to flowering, biomass, branching, leaf chlorophyll content, plant height, seed weight, and total grain yield. Thinning time influenced number of branches, plant height, above ground biomass and yield. Plant height increased between 32 and 39% for the thinned treatments. Early thinning increased biomass; thinning at the V8 stage increased biomass by 39% compared to the unthinned treatments. Magoye at 2.42 tons/ha had a higher yield, compared to Dina (2.37 tons/ha) and Sc Safari (1.76 tons/ha). Early thinning (T1) reduced yield by 28%. Soybean varieties used in this study exhibited significant vegetative plasticity. Reducing plant density especially in early vegetative phases reduced plant height, increased branching and biomass allocation to vegetative plant parts.**

**Key words:** Biomass, determinate, indeterminate, phenology, vegetative plasticity, yield, yield components.

## INTRODUCTION

Although biologists have always been aware that organisms respond differently to different conditions, environmental effects on phenotype were previously regarded as 'noise' obscuring 'true' expression of the genotype (Sultan, 1992). This led to the overlooking of the much more interesting aspect of plastic response to environmental variation (Sultan, 2003). Phenotypic responses to different environments may include highly

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specific developmental, physiological and reproductive adjustments that enhance function in these environments (Bradshaw, 1965).

Phenotypic plasticity has been described as the ability of a phenotype to be modified by the environment (Bradshaw, 1965). Nicoglou (2015) discussed the historical aspects of evolution of the plasticity concept, outlined emerging debates and the central role of Bradshaw in developing a model to explain the evolution of plasticity. Two forms of plasticity are recognized, morphological and physiological, each with different mechanisms, resource costs and ecological implications (Bradshaw, 1965; Murren et al., 2015). The first form is essentially meristematic in character and involves replacement of existing tissues by new plant parts with different phenotypic characteristics: it appears to represent a high-cost solution to a change in environment (Grime and Mackey, 2002). The second, physiological plasticity, occurs in differentiated tissues and is associated with visually imperceptible changes in properties brought about by reversible sub-cellular rearrangements: here the costs are lower and the response can be much more rapidly achieved than in morphological plasticity (Mooney and Gulman, 1979; Grime and Mackey, 2002; Larsen and Kershaw, 1975). It has been hypothesized that the two forms of plasticity have consistent associations with distinct sets of traits that coincide with particular habitats and ecologies (Grime, 1977; Grime et al., 1986). The importance of genetic inheritance in plant phenotypic responses in adapting to different environments has remained inconclusive and some workers have suggested that developmental processes of the organism itself act in conjunction with environmental influences to produce an integrated, adaptive phenotypes (Schmalshausen, 1986; Sultan, 1992).

Typically cultivated soybean comprises two crop development forms; the determinate types where vegetative and reproductive phases are well defined and do not overlap. The vegetative activity of the terminal bud ceases when it becomes an inflorescence. Indeterminate types, where flowering and vegetative phases overlap, the terminal bud continues vegetative activity during most of the reproductive flowering period (Zhang and Smith, 1999). With determinate forms, flower, pod and seed development are uniform throughout the plant and consequently seed maturity occurs over a short time range.

Conventional crop production practices require planting crops in well-defined regular spacing and plant density. Two general concepts are often used to explain the relationship between row spacing, plant density, and crop yield (Mellendorf, 2011; Shamsi and Kobraee, 2011). The first concept states that maximum crop yield can only be achieved if the crop community is able to produce sufficient leaf area to provide maximum light interception during reproductive growth. The second one, equidistant plant spacing maximizes yield because it minimizes interplant competition (Mellendorf, 2011; Shamsi and Kobraee, 2011; Wiggans, 1939). Plant density affects solar radiation interception capacity by the canopy.

The capacity of the crop to capture radiation throughout the crop cycle is closely associated with biomass production at harvest, and therefore, the magnitude of yield (Rondanini et al., 2017; Mataa et al., 2018). Additionally, light quality serves as a sensory cue for the adjustment of plant growth and development (Park and Runkle, 2017). Supra optimal planting density imposes competition for light on plants. Plants perceive the low R/FR ratios principally through the phytochrome B photoreceptor, which promotes the shade avoidance syndrome (SAS), a set of physiological responses that increase the elongation of vegetative structures such as stems and petioles, accelerates flowering, and reduces the number and size of seeds (Smith and Whitelam, 1997). When plant density increases, the reduction of photosynthetically active radiation (PAR) and blue photons partially induce overlapping SAS signaling pathways (Keller et al., 2011).

Due to the increasing importance of soybean in Zambia, new varieties are being developed (SCCI, 2013) and released but production recommendations have not changed to take advantage of emerging varieties variable phenotypic characteristics. The high cost of soybean seed necessitates the re-evaluation and optimization of planting densities recommendations. Soybean yield is considered a function of four basic factors, commonly called ‘yield components’, which include seed mass, number of seeds/pod, number of pods/plant, and number of plants per given area (Zhang and Smith, 1999).

Phenotypic plasticity has been observed in soybean crop communities in response to seeding rate and row spacing (Mellendorf, 2011). The mechanisms responsible for this yield compensation are not a fully understood subject. Additionally, the degree to which genotypes with different crop growth types exhibit phenotypic plasticity is unknown.

In an earlier study, we investigated the plasticity responses of a number of soybean varieties when subjected to different population density stresses (Sichilima et al., 2018). The findings showed planting density significantly influenced biomass allocation and grain yield. The objective of the current study was to determine if the stress duration or developmental stage at which planting density stress is imposed affects phenotypic plasticity responses and whether plants subjected to such stress can recover after stress removal. Additionally, the study tested the influence of genotype and crop growth type (determinate and indeterminate) on these plasticity responses. Plants subjected to supra-population density at planting were thinned to normal population density at different phenotypic stages so as to achieve variable stress durations. It is anticipated that findings from the study can help in optimizing crop production and optimal use of seed (and plant population) especially of new varieties.

## MATERIALS AND METHODS

### Study site

The study was conducted at the Seed Control and Certification Institute situated at 15° 32.772’ S and 28° 15.796’ E and elevation of 1246 m above sea level in Chilanga district of Lusaka Province of Zambia from December 2015 to April, 2016. Planting was done on the 21st of December, 2015 (which was within the recommended period in Zambia). The soils were a sandy-loam belonging to the Makeni series and classified as *Ultic Haplustalf.* Standard agronomical practices for growing soybean were used to raise the crop (Miti, 1995). At planting, a basal dressing fertilizer was applied at the rate of 20 kg N, 40 kg $P_2O_5$ and 20 kg $K_2O$ (D Compound) per ha (Miti, 1995). Harvest was done shortly after physiological maturity on 22nd April, 2016.

### Plant material

Three varieties were used; Dina, Magoye and Sc Safari (SCCI, 2013). Dina was obtained from MRI/Syngenta Seed Company, it is an indeterminate variety which is non-promiscuous. Magoye was obtained from Zamseed Company and is a promiscuous and determinate variety. Sc Safari is a determinate non-promiscuous variety from SeedCo (Z) Seed Company. The test varieties were chosen because of their high popularity and also due to having contrasting growth characteristics. According to the classification of the International Center of Tropical Agriculture (CIAT 1985), commonly used on dry beans, the growth habits can be classified as: (i) Type I: shrub determinate growth habit, the plants cease the vegetative growth after the insertion of the first floral bud; (ii) Type II: shrub indeterminate growth habit, plants continue their vegetative growth after flowering. They emit few branches and their branches do not emit guides and (iii) Type III: prostrate indeterminate growth habit, with well-developed branch ending in guides. The varieties used in this study were Type I (Magoye and Safari) and II (Dina).

### Experimental design and field layout

The trial was laid as a randomized complete block design arranged in a factorial design with two treatments (variety and thinning) replicated four times (Sokal and Rolfe, 1981). The varieties were planted at a planting density of 500,000 plants per hectare and thinned to 400,000 plants which is the recommended spacing in Zambia (Miti, 1995). Thinning was done at four developmental stages that represented key developmental transition stages: V1 (Full expansion of first trifoliate leaves- transition to autotrophic stage), V3 (Four nodes with 3 unfolded trifoliates), V6 (Seven nodes, with 6 unfolded- unifoliate, cotyledons senesced maximum branching stage) and R2 (Full bloom with an open flower at the top two nodes of the main stem) (Mc Williams, 1999; Zhang and Smith, 1999). An unthinned treatment was used as control, giving a total of 5 planting densities.

### Measured parameters

#### *Plant height*

Plant height was measured with use of a ruler at different growth stages, with the final one being at R6-R7 growth stage. At this point, the plant will have attained its full height and growth would have ceased (Casteel, 2011; Mc Williams, 1999).

#### *Number of branches*

The number of branches has a bearing on final yield obtained as pods tend to be borne on the branches. The total number of branches per plant was determined from the average of five randomly selected plants per treatment.

#### *Chlorophyll content*

Leaf chlorophyll content was determined by use of chlorophyll meter (Konica Minolta SPAD 502 Plus). The SPAD meter measures green color intensity in leaves *in vivo* and is for collecting large amounts of data on chlorophyll in the field within a short time non-destructively (Moe, 2012).

#### *Biomass*

Biomass was obtained by sampling 5 randomly selected plants per replication. The plants were carefully dug up and weighed to get fresh weight then dried at 65°C for 48 h then weighing them again to get dry weight.

#### *Yield*

After physiological maturity the pods were harvested and shelled, and seed dried under shade to a 15% moisture content. Yield was calculated as a function of base population, pod number, seeds per pod and seed weight (Casteel, 2011) at the harvestable moisture content of 15%.

#### *Seed weight*

Seed weights were obtained by counting 100 seeds in three replicates per treatment, weighing them to come up with a representative 100-seed weight.

#### *Days to 50% flowering*

The number of days was calculated from the time of plant emergence to when the plants reached 50% flowering and data was collected at the R1-R2 growth stage. The days to 50% flowering occurs at the time a plant has entered the reproductive phase (UPOV, 1998).

#### *Days to pod filling*

In soybean, full seed stage occurs at R6 growth stage and this stage is also known as the “green bean stage” marking maximum pod filling (Mc Williams et al., 1999). It was determined as the total number of days from emergence to this stage.

#### *Days to full maturity*

The number of days was thus calculated from emergence to R8. It is the stage when most of the leaves have lost their greenness and marks the plant’s whole growth period.

### *Number of pods per plant*

The number of pods per plant was determined by counting pods from a sample of five randomly sampled plants and expressed as the mean number of pods per plant. This was done at the R7-R8 growth stage when all the pods had fully formed and matured. The number of pods per plant is a significant factor in determining the plant yield (Casteel, 2011).

### *Number of seeds per plant*

The number of seed per plant was calculated by multiplying average of locules per pod and pods per plant. Like the number of pods per plant, the number of seeds per plant contributes to the determination of the final yield (Casteel, 2011).

### Data analysis

Data was analyzed using GenStat statistical package Version 12 (VSN, 2009). The data was subjected to analysis of variance and where significant treatment effects were detected, mean separation was done using the least significant difference (LSD).

## RESULTS

Table 1 summarizes effect of treatments on vegetative and reproductive parameters. Genotype had highly significant effect on most of the parameters measured; days to flowering (length of vegetative phase), biomass, branching, leaf chlorophyll content, plant height, seed weight, and total grain yield. Time of thinning exerted effect on number of branches, plant height, above ground biomass and yield.

### Effect on vegetative parameters

### *Plant height*

The time of thinning was done had a significant effect on plant height (Table 2 and Figure 1). Dina had significantly taller plants compared to Magoye and Sc Safari ($p \leq 0.01$). Unthinned plants or those thinned late (T3 and T4) were significantly taller than thinned early (T1 or T2). The increase in plant height was between 31 and 39% relative to the unthinned treatment. This difference was more evident in the early growth stage (V4).

### *Biomass*

Effects of treatments on biomass are shown in Table 1 and Figure 1. Increasing plant density increased biomass. The early thinned treatments exhibited the highest increase in biomass, the increase was significant in the late vegetative stage (V8) where the unthinned treatments had 13.3 g dry matter per plant, 18 g for the T1 thinning, representing a 35% increase. The increase in biomass decreased with delayed thinning (Figure 1).

### *Branching*

There were no differences in the number of branches among the genotypes (Table 2 and Figure 1). The number of branches increased with thinning, the difference was significant between the non- thinned treatment and those treatments thinned at V3 stage ($p \leq 0.05$). There were no significant differences in number of branches among the other thinning treatments.

### *Chlorophyll content*

The chlorophyll content was not affected by thinning stage but there were differences among genotypes (Table 1 and Figure 2). Chlorophyll content was higher in the variety Sc Safari compared to the other two genotypes ($p \leq 0.05$). There were differences in chlorophyll content among genotypes, however, these differences did not translate in increased productivity or yield as the genotype that had higher chlorophyll (Sc Safari) had low yield relative to the other two.

### Effects on reproductive parameters

The effects of treatments on reproductive parameters are shown in Table 2 and Figure 2. Generally, thinning had less effect on reproductive parameters.

### *Days to 50% flowering*

There were differences among the genotypes in the duration of the period to flowering. Sc Safari at 51.7 had the shortest days to flowering and Dina the longest at 66.4 days. Time of thinning did not affect days to flowering. The number of days to flowering is important because it determines the period available for the plant to synthesize and accumulate photo assimilates and these are used for plant development and ultimately yield. The genotype that had the longest days to flowering duration also had the highest yield.

### *Locules per pod*

Genotype had a significant effect on locules per pod. Dina had significantly more locules per pod compared to Sc Safari. Magoye was intermediate. Time of thinning did not affect number of locules per plant.

### *Number of pods per plant and seeds per plant*

The number of pods per plant varied between 20.5 and 34.3, Magoye had significantly more pods per plant and seeds per plant. There was a trend of number of pods per

**Table 1.** Summary of analysis of variance of planting density and genotype effects on vegetative and reproductive parameters of soybeans.

| Parameter$^{Z}$ | 100 SDWT | 50%DF | BWT1 | BR | CLV4 | CLV6 | DPF | LPP | NSP | PPD | PHV4 | PHR8 | RDW | BWT2 | YLD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variety (V)** | *** | *** | *** | ns | ** | *** | *** | ** | *** | *** | *** | *** | ns | *** | ** |
| **Thinning (T)** | ns$^{y}$ | ns | ns | ** | ns | ns | ns | ns | ns | ns | ** | ns | ns | ** | ** |
| **V × T** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **CV%** | 3.9 | 0.2 | 5.8 | 9.3 | 4.4 | 1.8 | 0.1 | 3.1 | 6.2 | 3.9 | 5.6 | 6.6 | 4.7 | 4.0 | 8.8 |

$^{z}$100 seed weight (100SDWT); Days to 50% flowering (50%DF); Biomass dry weight/plant at R3 (BWT1); No. of branches/plant (BR); Chlorophyll content at V4 (CLV4); Chlorophyll content at V6 (CLV6); Days to pod filling (DPF); No. of locules/pod (LPP); No. of seeds/plant (NSP); No. of pods/plant (PPD); Plant height at V4 (PHV4); Plant height at R8 (PHR8); Root dry weight (RDW); Biomass weight per plant at R8 (BWT2);Grain yield/hectare (YLD). $^{y}$Level of significance; ns, **, *** denoting non-significant, significant at $p \leq 0.05$ and $p \leq 0.001$.

**Table 2.** Effect of planting density on vegetative parameters in selected soybean genotypes

| Source of variation | | Chlorophyll content$^{z}$ | Plant height | | Branches per plant | Biomass$^{y}$ | Days to Flowering |
|---|---|---|---|---|---|---|---|
| | | | V4 | R8 | | | |
| | Dina | 35.6$^{aw}$ | 22.5$^{b}$ | 85.0$^{b}$ | 2.89$^{a}$ | 18.7$^{b}$ | 66.4$^{b}$ |
| Variety (V) | Magoye | 33.5$^{a}$ | 18.5$^{a}$ | 81.2$^{b}$ | 3.35$^{a}$ | 15.1$^{a}$ | 64.7$^{b}$ |
| | SC Safari | 38.8$^{b}$ | 19.7$^{a}$ | 57.5$^{a}$ | 3.07$^{a}$ | 13.8$^{a}$ | 51.7$^{a}$ |
| | T0 | 35.7$^{a}$ | 20.0$^{ab}$ | 73.0$^{a}$ | 2.51$^{a}$ | 13.3$^{a}$ | 61.0$^{a}$ |
| | V1 | 36.2$^{a}$ | 19.9$^{ab}$ | 76.8$^{a}$ | 3.31$^{ab}$ | 18.0$^{b}$ | 60.6$^{a}$ |
| Thinning time$^{y}$ | V3 | 36.4$^{a}$ | 19.0$^{a}$ | 74.1$^{a}$ | 3.49$^{b}$ | 17.7$^{b}$ | 61.1$^{a}$ |
| | V6 | 35.3$^{a}$ | 21.2$^{b}$ | 73.2$^{a}$ | 3.36$^{ab}$ | 15.1$^{ab}$ | 61.1$^{a}$ |
| | R2 | 36.2$^{a}$ | 21.1$^{ab}$ | 75.7$^{a}$ | 2.84$^{ab}$ | 15.3$^{ab}$ | 60.9$^{a}$ |
| Factor significance$^{v}$ | | | | | | | |
| Variety | - | *** | *** | *** | *ns* | *** | *** |
| Planting density | - | *ns* | ** | *ns* | ** | ** | *ns* |

$^{z}$Leaf chlorophyll content given as SPAD meter reading; $^{y}$Total biomass measured at V3; $^{x}$Thinning time T0 non thinned-maintained at 500, 000 plants per hectare; V1, V3, V6 and R2, respectively thinned from 500,000 to 400,000 plant population at V1, V3, V6 and R2 developmental stages. $^{w}$Figures followed by same letter denote no significant difference. $^{v}$Level of significance ns, **, *** denoting non-significant, significant at $p \leq 0.05$ and at $p \leq 0.001$.

plant and seeds per plant increasing with thinning, but this trend was not statistically significant (Figure 2) and was more evident in Magoye. Days to pod filling and days to full maturity were also not affected significantly by genotype or thinning time.

### *Yield*

Planting density had significant effects on yield

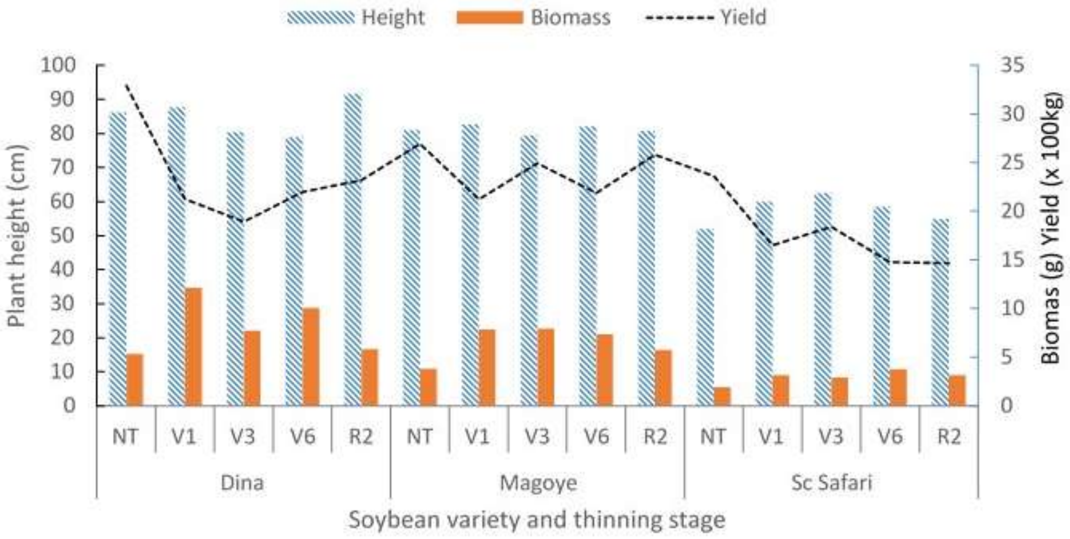

**Figure 1.** Changes in vegetative parameters (plant height and total biomass) and grain yield in soybeans varieties thinned at various growth stages. NT, non-thinned-(maintained at 500,000 plants/ha); V1, V3, V6, and R2 being soybean developmental stages at which the plants were thinned from 500,000 to 400,000 plant population.

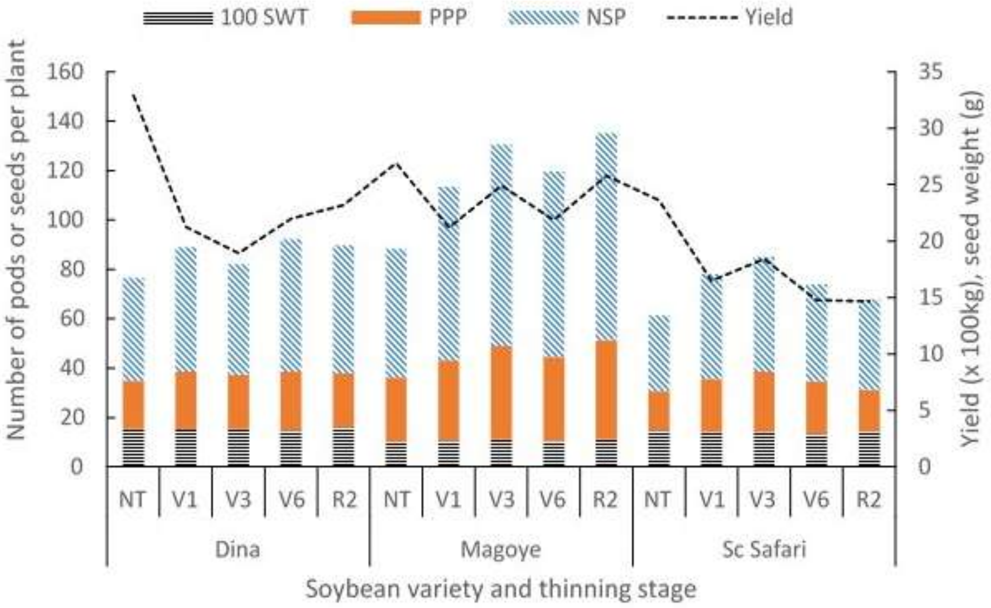

**Figure 2.** Changes in reproductive parameters (100-seed weight, pods per plant (PPP), number of seeds per plant (NSP) and grain yield in 3 soybeans varieties thinned at various growth stages. NT, non-thinned-(maintained at 500,000 plants per hectare); V1, V3, V6, and R2 being soy bean developmental stages at which the plants were thinned from 500,000 to 400,000 plant population.

shown in Table 3 and Figure 2. Yield was the highest in unthinned treatments and tended to decline with thinning. The late thinning (at V6 and R2) yields were similar to the unthinned treatment (NT). The decline in yield was the most significant with earlier thinning compared to late thinning with an average reduction in yield of 0.8 ton/ha between the unthinned treatments and the thinned ones. In terms of genotype, Magoye, a determinate variety had significantly higher yield compared to the other 2 varieties across all treatments, although Dina had the highest yield

**Table 3.** Effect of planting density on reproductive parameters in selected soybean genotypes.

| Source of variation | | Days to 50 % Flowering | Locules/Pod | Pods/Plant | Seeds/Plant | Seed weight | Grain yield |
|---|---|---|---|---|---|---|---|
| Variety (V) | Dina | $66.4^{c\ z}$ | $2.2^{b}$ | $22.3^{a}$ | $48.4^{a}$ | $15.2^{c}$ | $2.37^{b}$ |
| | Magoye | $64.7^{b}$ | $2.1^{ab}$ | $34.3^{b}$ | $72.6^{b}$ | $10.6^{a}$ | $2.42^{b}$ |
| | SC Safari | $51.7^{a}$ | $1.9^{a}$ | $20.5^{a}$ | $39.1^{a}$ | $14.0^{b}$ | $1.76^{a}$ |
| Thinning time[y] | T0 | $61.0^{ax}$ | $2.0^{a}$ | $20.7^{a}$ | $41.3^{a}$ | $13.3^{a}$ | $2.8^{b}$ |
| | V1 | $60.6^{a}$ | $2.1^{a}$ | $25.9^{a}$ | $54.3^{a}$ | $13.2^{a}$ | $2.0^{a}$ |
| | V3 | $61.1^{a}$ | $2.0^{a}$ | $28.3^{a}$ | $57.6^{a}$ | $13.4^{a}$ | $2.1^{ab}$ |
| | V6 | $61.1^{a}$ | $2.1^{a}$ | $26.5^{a}$ | $55.9^{a}$ | $12.8^{a}$ | $1.9^{a}$ |
| | R2 | $60.9^{a}$ | $2.2^{a}$ | $26.5^{a}$ | $57.5^{a}$ | $13.7^{a}$ | $2.1^{ab}$ |
| Factor significance[v] | | | | | | | |
| Variety | | *** | *** | *** | *** | *** | *** |
| Planting density | | *ns* | *ns* | *ns* | *ns* | *ns* | ** |

[z]Figures followed by same letter denote no significant difference. [y]Level of significance ns, [x]Thinning time T0 non thinned-maintained at 500, 000 plants per hectare; V1, V3, V6 and R2, respectively thinned from 500,000 to 400,000 plant population at V1, V3, V6 and R2 developmental stages. [w]Figures followed by same letter denote no significant difference. **, *** denoting non- significant, significant at $p \leq 0.05$ and at $p \leq 0.001$.

in the unthinned treatments.

## DISCUSSION

### Effects of treatments on vegetative development

The results obtained showed significant treatment effects on vegetative parameters especially thinning time on plant height and branching and biomass accumulation. An ecologically important yet environmentally labile aspect of plant development is the ability to vary the proportion of biomass allocated tissues such as roots, leaves, stems and reproductive structures (Bazzaz, 1996). This allows plants to adaptively enhance access to a specific resource in short supply. This altered partitioning is described as allocational plasticity (Sultan, 2003). Plasticity for biomass allocation to plant tissues is a major means of plant adjustment to the environment. It has been suggested that developmental shifts due to changes in sink; source relationships are mediated through changes in plant growth regulators whose resource costs are minute (Sultan, 1992; Bradford and Hsiao, 1982). Although thinning stage affected biomass allocation, this biomass, however, was directed towards vegetative tissues and not grain yield.

### Effects on leaf chlorophyll content

Chlorophyll is an important pigment for light capture and therefore the amount of chlorophyll is critical in increasing the photosynthetic rate and ultimately accumulation of photoassimilates. High plant densities induce shade effects that can affect leaf chlorophyll content (Mataa and Tominaga, 1998) but this effect was not observed in this study. One possible explanation could be that since soybeans are a leguminous plant that fixes nitrogen-a key component of chlorophyll- and also because the plant density stress developed gradually, the plants were able to adjust and correct any chlorophyll imbalance. As noted earlier by Park and Runkle (2017), De Luca and Hungria (2014), and Ballaré et al. (1990), within a crop community, plants growing under a canopy not only experience a reduction in the amount of irradiance, but also a reduction in the quality of light as chlorophyll preferentially absorbs red light (R) and reflects far-red (FR) light. Many plant species show a range of tolerance to variations in light intensity and this is achieved through morphological, physiological and allocation plasticity (Sultan, 1992). Photosynthetic plasticity appears to incur little physiological or structural cost (Gross, 1984). Typically, leaves that develop in low-light show changes in chloroplast structure that enhance light harvesting capacity and this is not metabolically costly (Mooney and Gulman, 1979). The results showed that differences in chlorophyll content genotypic were due to genotype but not plant density. It can be postulated that because effects of crop density did not elicit sufficient environment effects to cause significant changes in chlorophyll content, the effects on leaf photosynthesis were minimal.

### Effects on reproductive development

The number of locules and seed weight are important determinants of grain yield and it was observed that Dina with slightly more and larger heavier seeds and more locules, also exhibited significantly higher yield. Thinning increased branching and canopy biomass but that biomass was directed towards vegetative tissues and not seed. Thinning (and resultant decreased plant density) did not have significant effects on many yield components possibly because flower induction phase occurs much earlier in the development cycle and by the time thinning was done the process had already terminated (Zhang and Smith, 1999). The yield decline noted in the very early thinned treatment could indicate that the recommended plant population of 400, 000 plants per hectare is lower than optimum and these test varieties can withstand a higher plant density and cause significant yield increase.

### Influence of growth characteristics

Changing crop population density modifies resource availability to individual plants. The ability to use these resources depends on plant strategies that induce vegetative and reproductive plasticity (Rondanini et al., 2017; Roiloa et al., 2014). Plasticity responses to the environment are dynamic and vary with time (Murren et al., 2015). It has been postulated that modular organisms (plants with indeterminate growth characteristics) may compensate for any negative impacts through flexible addition of new modules (Murren et al., 2015). In general, plants with indeterminate growth habit have higher total cycle and height of plants in relation to the determinate growth habit (Velho et al., 2018). Although, the present study included varieties that had contrasting growth characteristics (determinate and indeterminate), no differences were observed between the two different growth categories except for yield. It was interesting to note that whereas in this study we varied the duration and/or timing of population density stress, similar results were obtained in a related study where we had a constant population stress duration (Sichilima et al., 2018).

## Conclusion

The soybean varieties used in this study exhibited significant vegetative plasticity. Reducing plant density especially in early vegetative phases reduced plant height, increased branching and biomass allocation to vegetative plant parts. However, these effects did not always increase yield. The growth type of the variety tested, whether determinate or indeterminate, did not appear to exert any significant influence on plasticity.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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*Full Length Research Paper*

# Soil hydro-physical attributes under management practices for pineapple genotypes cultivation

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**Pineapple is a rustic species but may respond positively to conservation practices of soil management. This work aimed to evaluate the physical properties of the soil under conservation management practices for the cultivation of pineapple genotypes. The treatments consisted of four varieties of pineapple, two levels of gypsum (0 to 4 t ha$^{-1}$), two levels of management (with and without cover crop - millet), and two soil layers (0 -0.05 and 0.05 - 0.20 m). A randomized complete block design was used, with four replications and treatments arranged in a 4 x 2 x 2 x 2 factorial scheme, resulting in 128 plots. Soil samples were collected in volumetric corer of 100 cm$^3$. The water retention curve of the soil was obtained using the Van Genuchten model. By model fit, residual moisture values (θr), saturation moisture (θs), inflection point (θfwc), S index and available water capacity (AWC) were obtained. Also, there was a significant effect of layer and genotype on the properties studied. The combination of gypsum 4 t ha$^{-1}$ (G4) and millet significantly reduces the value of θr and increases water availability (AWC).**

**Key words:** *Ananas comosus*, available water capacity, Red-Yellow Latosol, cover crop.

## INTRODUCTION

Pineapple, *Ananas comosus* (L.) Merrill, var. *comosus* is a perennial herbaceous fruit belonging to the Bromeliaceae family and is thought to have originated in lowland South America, possibly in the southwest of

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Brazil (Santana et al., 2013; Ming et al., 2016). The other possibility is that *A. comosus* var. comosus has been domesticated in the Guiana Shield (Coppens d'Eeckenbrugge and Duval, 2009). Particular attributes of pineapple fruit associated with asexual reproduction, wide adaptation to different environments, and the beginning of its agriculture in the South America at Amazon region suggest that the pineapple domestication Occurred between 6,000 and 10,000 ago (Coppens d'Eeckenbrugge and Duval, 2009).

In Brazil, two main breeding programs in pineapple are being conducted, in which one is coordinated by the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), located in Cruz das Almas (BA), which has already released varieties as 'BRS Imperial', 'BRS Ajubá' and 'BRS Vitória' and the other one is being conducted by the Instituto Agronômico de Campinas (IAC) that has developed 'IAC Fantástico' and 'Gomo de Mel' varieties (Viana et al., 2013).

The great success of pineapple crop in Brazil is due to the wide adaptability of species in tropical and subtropical areas, high rusticity, easy propagation, and especially great fruit acceptability (Crestani et al., 2010). Species, especially commercial varieties derived from plant breeding programs, shows response to chemical soil improvements (Guarçoni and Ventura, 2011).

Even as the chemical conditions and the good physical conditions also benefit the pineapple crop, particularly those conditions related to soil, as well as water availability, which responds positively to the productivity and fruit quality (Santana et al., 2013). According to Dexter (2004), when soil physical quality is improved indirectly, improvements in biological and chemical conditions occur, since these soil quality aspects are mutually dependent. There are many symptoms regarding poor soil quality such as low aeration, low water infiltration and reduced root system, which reflects deterioration of soil structure (Dexter, 2004; Krebstein at al., 2014).

However, there are other indicators of soil physical quality as well as water retention curve, hydraulic conductivity, porosity, inflection point and soil water retention characteristics that make evaluation process of this quality simple, faster and less complex (Silva et al., 2014).

Proper soil management is crucial to crop success, because although pineapple may be a rough and resistant plant, the knowledge of soil physical and chemical characteristics as well as intervention for deviation correction is critical. Therefore, this study is aimed at evaluating soil hydro-physical attributes under different management practices for cultivation of pineapple genotypes.

## MATERIALS AND METHODS

The experiment was conducted in the experimental area of the Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso, Campus Cáceres - Prof. Olegário Baldo, located in Cáceres - MT, with average coordinates of 16° 7'50"S and 57°41'41"W and altitude of 120 m. The annual average temperature is 26.24°C; the total annual rainfall is 1.335 mm, with the period of highest average rainfall concentration occurs from December to March and the largest dry season occurring from June to August; the average potential evapotranspiration is 1.650 mm (Neves et al., 2011). Soil from experimental area is classified as Red-Yellow Latosol (LVA), Sandy texture, with flat topography.

The pineapple cultivars, Pérola, Smooth Cayenne, IAC - Fantástico and Imperial were used in this study. In addition, soil correction levels were performed without gypsum (G0) and with application of gypsum at dose of 4 t $ha^{-1}$ (G4), adapted from Cantarutti et al. (1999). The management were performed with and without millet cover crop.

A randomized block design in factorial 4x2x2x2 were used, totaling 32 treatments and four replications. Each plot consisted of 20 plants arranged in double row planting, 0.30 x 0.40 x 0.90 m spaced, totaling 1,280 plants. The average length of seedlings used in planting was 44.3, 44.6, 22.9, and 23.6 cm for Pérola, Smooth Cayenne, IAC Fantástico and Imperial varieties, respectively.

Gypsum was distributed as continuous thread in planting furrow at a layer of 15 cm, and then covered with a thin layer of soil, following fertilization and planting itself. Gypsum moisture was determined for quantity correction to be applied at a dose of 4 t $ha^{-1}$. The cover crop with millet was grown between lines of double rows, with 2 g $m^{-1}$ of seed. Millet management was performed with cuts of 0.10 m above the soil when plant reached a height of 60 cm.

For soil sample collection, the same experimental design was used. A sample per plot with preserved structure was collected, positioned 0.10 to 0.20 m from plant base, using volumetric core of approximately 100 $cm^{3}$ (0.048 m in diameter by 0.049 m in height) in 0.0 to 0.05 m and 0.05 to 0.20 m layers in each experimental unit.

For the soil water retention curves (WRC) determination, samples contained in core were first saturated and posteriorly subjected to metric tension of 1, 2, 4, 6, 8 and 10 kPa using porous plate funnels (Haines, 1930) of 33, 66, 100, 300, 1,500 kPa in Richards Chambers (Richards, 1965). After reaching water equilibrium at each tension, samples were weighed and subjected to the next tension, constituting curve method by drying. After the last tension, samples were dried at 105±2ºC for 24 h to determine soil water content ($\theta_r$) (Teixeira et al., 2017).

The model proposed by Van Genuchten (1980) was adjusted to the experimental data of water retention in each experimental unit. The data were drawn by adjustment procedures of nonlinear model of R software and the values of residual moisture ($\theta_r$) and saturation moisture ($\theta_s$) were obtained.

For the adjustment, the following parameterization of Van Genuchten model was considered (Equation 1):

$$\theta(x)=\theta r+\frac{\theta s\text{-}\theta r}{(1+\exp\backslash\{n(\alpha+x)\backslash\})1\text{-}1/n} \qquad (1)$$

Where $\Theta(x)$ is soil moisture ($m^{3}\ m^{-3}$), *x* the log in 10 base of applied matricial tension (kPa), $\theta_r$ is residual moisture (inferior asymptote), $\theta_s$ is the saturation moisture (superior asymptote), while *α* and *n* are empirical parameters of water retention curve form. Once values of these terms are known, S index (S) (Equation 2), tension at inflection point of curve (I) (Equation 3) and moisture at inflection point (θI) (Equation 4) are obtained:

$$S= -n\cdot\frac{\theta s-\theta r}{(1+1/m)^{m+1}} \qquad (2)$$

**Table 1.** Summary of variance analysis for study variables in four cultivars of pineapple, two levels of agricultural gypsum, two soil cover levels and two study layers: $\theta_r$ (residual moisture), $\theta_s$ (saturation moisture), S index, Inflection Point ($\theta_{fwc}$), Available water capacity (AWC).

| Means squares | | | | | | |
|---|---|---|---|---|---|---|
| **Treatments** | **GL** | **$\theta_r$** | **$\theta_s$** | **ÍNDICE S** | **$\theta_{fwc}$** | **AWC** |
| Cover | 1 | 0.0000014 $^{NS}$ | 0.004879 $^{NS}$ | 0.0016143 $^{NS}$ | 0.00764 $^{NS}$ | 0.0008848 $^{NS}$ |
| Gypsum | 1 | 0.0000746 $^{NS}$ | 0.001788 $^{NS}$ | 0.0000810 $^{NS}$ | 0.00142 $^{NS}$ | 0.0009368 $^{NS}$ |
| Layer | 1 | 0.0014229** | 0.075827** | 0.0102480 $^{NS}$ | 0.00142** | 0.0156296** |
| Variety | 3 | 0.0002433$^{NS}$ | 0.000386 $^{NS}$ | 0.0023324 $^{NS}$ | 0.01396 $^{NS}$ | 0.0004352 $^{NS}$ |
| Cover crop:gypsum | 1 | 0.0044550$^{**}$ | 0.006721 $^{NS}$ | 0.0000115 $^{NS}$ | 0.17452* | 0.0059612** |
| Cover crop:layer | 1 | 0.0000297$^{NS}$ | 0.003100 $^{NS}$ | 0.0046337 $^{NS}$ | 0.02022 $^{NS}$ | 0.0006589 $^{NS}$ |
| Cover crop:variety | 3 | 0.0005295.* | 0.003247 $^{NS}$ | 0.0046337 $^{NS}$ | 0.08687.* | 0.0008682 $^{NS}$ |
| Gypsum: Layer | 1 | 0.0002396$^{NS}$ | 0.003934 $^{NS}$ | 0.0018293 $^{NS}$ | 0.05364 $^{NS}$ | 0.0008682 $^{NS}$ |
| Gypsum:Variety | 3 | 0.0000978$^{NS}$ | 0.001158 $^{NS}$ | 0.0069133 $^{NS}$ | 0.06601 $^{NS}$ | 0.0002086 $^{NS}$ |
| Layer:Variety | 3 | 0.0000455$^{NS}$ | 0.010138* | 0.0036408 $^{NS}$ | 0.15943** | 0.0038503** |
| Residue | 108 | 0.0002144 | 0.002576 | 0.0036408 | 0.03506 | 0.0007084 |

**, * and $^{ns}$: significant at 1% and 5% probability and not significant respectively.

$$I = -\alpha - \log(m)/n \qquad (3)$$

$$\theta I = \theta\ (x=I) \qquad (4)$$

Where *S* is the rate at inflection point, a parameter which is considered an indicator for soil physical quality evaluation as well as θ*I* that corresponds to the tension log at inflection point of soil water retention curve (Dexter, 2004). The moisture corresponding to the tension at inflection point is represented by θI. Field water capacity ($\theta_{fwc}$) was considered moisture in θI ($\theta_{fwc}$=θI). Permanent wilting point ($\theta_{pwp}$) was obtained in water content in $\theta_r$ and in 1500 kPa potential. Available water capacity (AWC) was calculated by the difference between $\theta_{fwc}$ less $\theta_{pwp}$.

All data were subjected to analysis of variance (ANOVA) at 1 and 5% probability, and means compared by t test using the R program (R Core Team, 2015).

## RESULTS AND DISCUSSION

Significance for layer factor was observed in relation to almost all the variables, except for S index. Regarding residual moisture ($\theta_r$) and available water capacity (AWC), positive effect was observed in relation to cover × variety interaction; in relation to cover × gypsum interaction, there were significance to saturation moisture ($\theta_s$), residual moisture ($\theta_r$), moisture at inflexion point ($\theta_{fwc}$) and available water capacity (AWC). For $\theta_s$, AWC and $\theta_{fwc}$, there was interaction in relation to layer × variety (Table 1).

Significant difference was observed for $\theta_r$ results in the comparison of 0.0-0.05 and 0.05-0.2 m layers. In the first layer, the mean was 0.09 $m^3\ m^{-3}$ and at 0.05-0.2 m layer was 0.08 $m^3\ m^{-3}$ (Figure 1A). The increase in $\theta_r$ decreases the AWC to the plants. In the absence of the cover crop, the gypsum increased the residual moisture; however, the effect of the gypsum was inverse in the presence of the cover crop. The combination of cover crop and gypsum was positive to decrease $\theta_r$ and contribute to the increase of AWC (Figure 1B).

In interaction split of gypsum × cover crop factors, $\theta_r$ significance of G4 in relation to G0 was observed in the absence of cover crop and reverse in cover presence. For treatments with G0, the means were 0.085 and 0.097 $m^3\ m^{-3}$ with and without cover respectively. For G4 values, it was 0.085 and 0.098 $m^3\ m^{-3}$ with and without cover crop, respectively (Figure 2). It is observed that gypsum effect is conditioned by cover crop, and its desired effect of reducing $\theta_r$ stands out only in the presence of cover crop. The gypsum × millet combination, besides reducing $\theta_r$ provides other advantages such as enrichment of Ca, S and input of organic matter in soil.

It was found that for $\theta_s$, significant effect was only observed for the interaction of variety × layer factors. For Imperial, Pérola and Smooth Cayenne varieties, $\theta_s$ was significantly higher in the 0.0 to 0.05 m layer with values of 0.393, 0.409 and 0.399 $m^3\ m^{-3}$, while in the 0.05-0.2 m layer, values were 0.353, 0.327 and 0.331 $m^3\ m^{-3}$, respectively. For IAC Fantástico variety, there was no significant difference between studied layers, with values of 0.374 and 0.372 $m^3\ m^{-3}$ for 0-0.05 and 0.05-0.2 m, respectively (Figure 2).

In a study on the row and between rows of coffee trees, as well as combined mixture of gypsum and organic material from brachiaria between rows, significant increase in $\theta_s$ value were observed (Serafim et al., 2013). This analogy can be used to compare layers 0-0.05 and 0.05-0.2 m, where there is greater accumulation of organic material on surface. This is not the case for IAC Fantástico whose growth was slowly leaving soil permanently exposed with the adverse effects of this exposure.

Regarding tension at inflection point (I), it was observed significance for layer × varieties interaction in which the Imperial Pérola e Smooth Cayenne varieties had higher

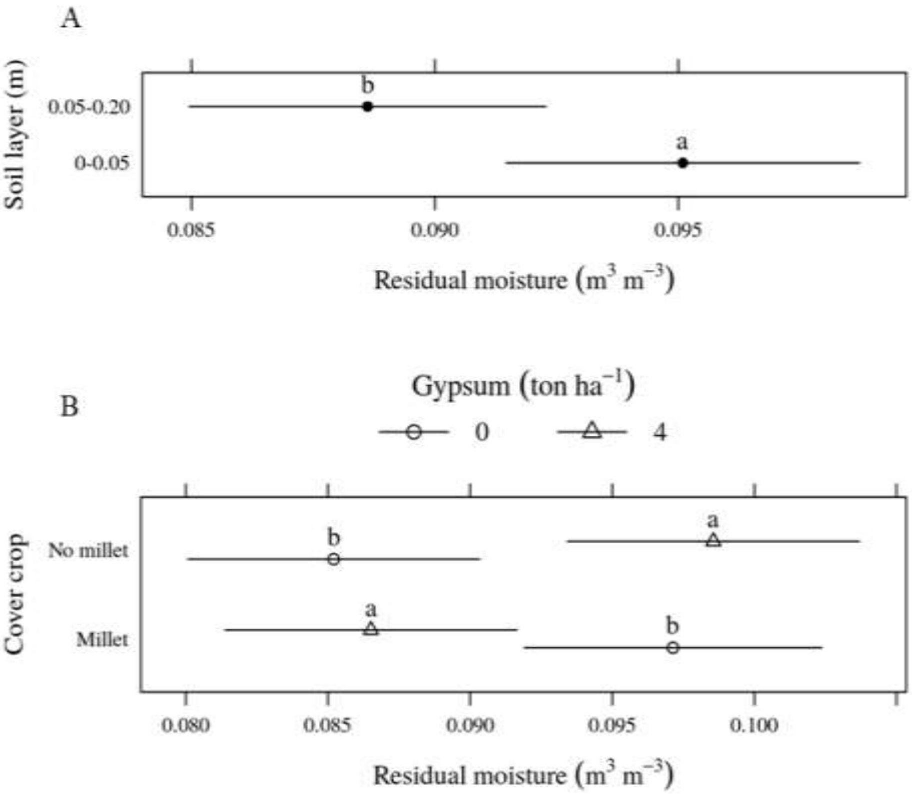

**Figure 1.** Residual moisture (1500 kPa) in the layers of 0.0-0.05 and 0.05-0.2 m [A] and in the conditions with and without cover crop for the two levels of gypsum (G0 and G4) [B].

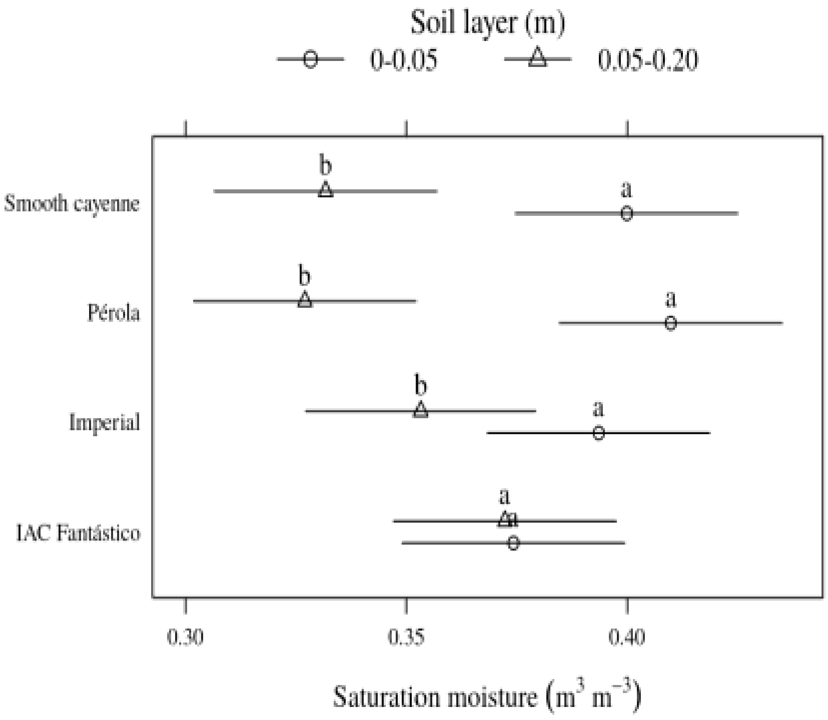

**Figure 2.** Saturation moisture for layers 0.0-0.05 and 0.05-0.2 m according to pineapple varieties.

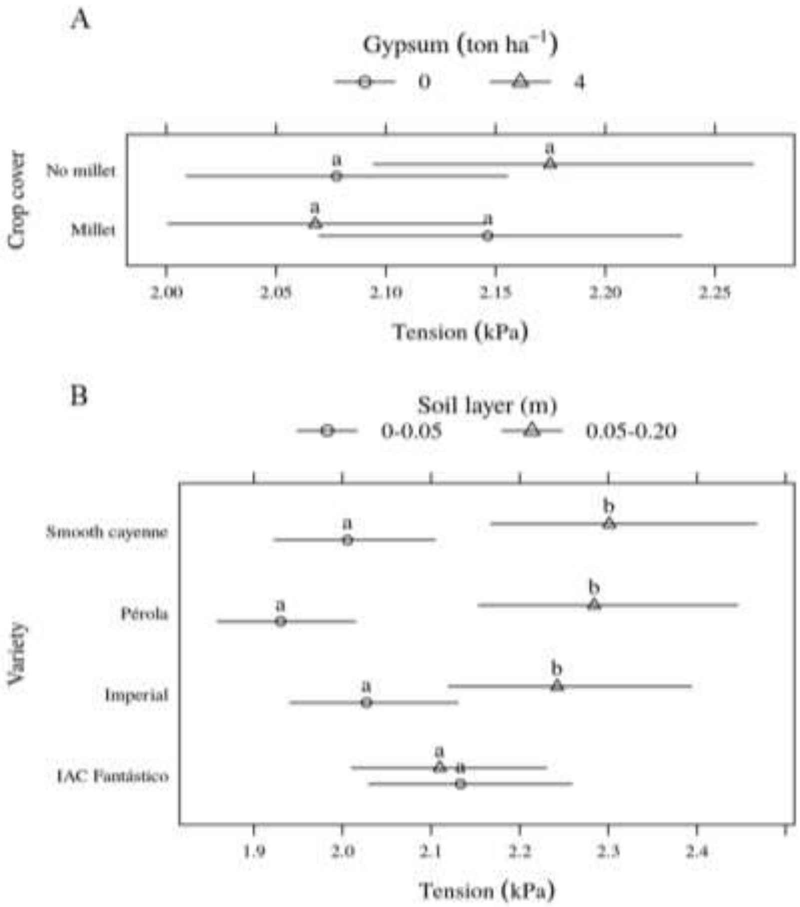

**Figure 3.** Tension values (kPa) corresponding to inflection point ($\theta_I$) for the interaction doses gypsum x millet [A] and tension values (kPa) corresponding to inflection point ($\theta_I$) at 0.0-0.05 and 0.05-0.2 m layers and at different pineapple varieties [B].

mean in surface layer with values of 1.41, 1.21 and 1.43 kPa, in relation to 0.05 to 0.2 m layer, which values were 1.23, 1.21 and 1.20 kPa, respectively. On the other hand, for IAC Fantástico variety, there was no significant difference in the studied layers (Figure 3B).

In the interaction of gypsum × cover factors, there was no significant difference for $\theta_I$ in the presence of G4 in relation to G0 for treatments with and without millet crop cover in which means with millet were 1.37 and 1.30 kPa for G4 and G0 respectively, and without millet values were 1.28 and 1.36 kPa for G4 and G0 respectively (Figure 3A). The lack of significant difference between management systems for potential or soil moisture in θfwc and θpwp is described by Rocha et al. (2015). The θfwc results from the complex interaction between clay content, structure, density and soil organic carbon, whose impact of the change of these factors on soil moisture in θfwc may be delayed for longer periods than one year. The θpwp is strongly related to the clay content which is not affected by management systems.

According to Silva et al. (2014), the inflection point of curve marks the division between the two distinct pores classes, analogous to macro and micropores. Usually, lower values on the surface are expected and assume greater potential values in subsurface layers with lower organic matter content. Ferreira and Marcos (1983) also found corresponding potential values to the inflection point less than 6 kPa by evaluating different Latosols. This behavior may be associated with high porosity of these soils, due to its granular type structure of high macroporosity as well as high amount of micropores, responsible for water retention in soil at field water capacity.

For the AWC, there was significance to layer × variety interaction, where Imperial, Pérola and Smooth Cayenne varieties had a significant higher effect in 0.0-0.05 m layer, with values of 0.160, 0.165 and 0.160 $m^3 m^{-3}$ compared to 0.05-0.2 m layer, where values were 0.140, 0.125 and 0.125 $m^3\ m^{-3}$ respectively (Figure 4A). The IAC Fantástico variety showed no significant difference in the studied layers, with values of 0.146 and 0.155 $m^3\ m^{-3}$ compared to 0-0.05 and 0.05-0.2 m layers, respectively

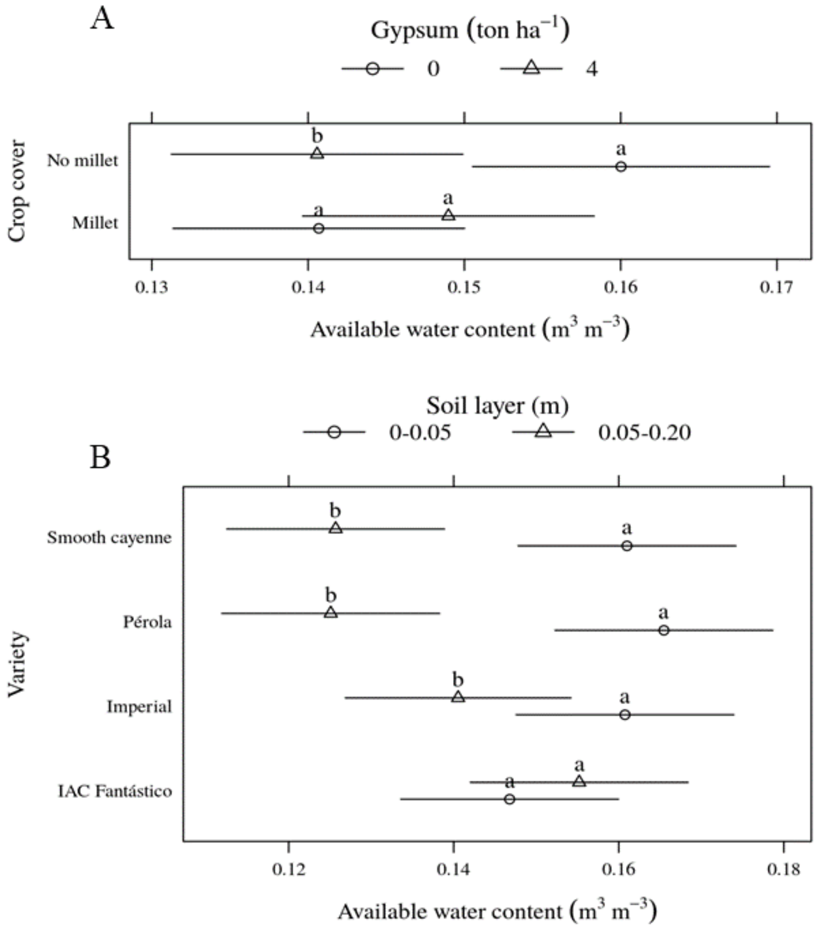

**Figure 4.** Available water content (AWC) compared to gypsum x cover crop [A] and available water content compared to layer x variety interaction [B].

(Figure 4B). The difference observed between layers in this work, was amplified by millet cover crop which increased organic matter in the first layer compared to the second. As already described, the non-closure of culture in IAC Fantástico, accelerated decomposition cycle, reducing C accumulation in surface and profile.

The AWC indicates the soil ability to store and provide water that is available to the roots. Despite not consider the dynamics of soil-water-plant-atmosphere interrelations (Silva et al., 2014), this concept has distinct practical importance for water balance, dry climate, setting of planting period, agricultural zoning, and specially in irrigation projects, which becomes a great important parameter in land use planning.

There was no significant interaction between gypsum × cover crop for the variable AWC. Regarding treatment without millet, there was significant difference of AWC for G4 and G0 in which means were 0.140 and 0.160 m$^3$ m$^{-3}$ for G4 and G0, respectively (Figure 4). In this variation source there is grouping of two studied layers, which may have diluted the effect of the treatment, especially residue which was deposited only on surface. This result differs from results of Serafim et al. (2012) and Silva et al. (2014), who shows the positive effect of cover crop and agricultural gypsum on increased AWC in coffee area, however, for longer period of conservation management.

AWC values obtained in this study are high, even in the most unfavorable situation, as described by Serafim et al. (2013) in Latosol and Cambisol, and Fidalski et al. (2013) in Quartz Neosol. This existing feature in soil, complicate the detection of positive effects of gypsum or cover crop (millet), within the study period of this study.

## Conclusions

All the pineapple varieties except IAC Fantástico had better indexes in the 0.0-0.05 m layer for $\theta$r, $\theta$s, θfwc and AWC physical attributes compared to 0.05 – 0.20 m layer, except for IAC Fantástico variety. The gypsum 4.0 t.ha$^{-1}$ (G4) and cover with millet combination contributed to increased water availability (AWC) by reducing tension value in θfwc and reduced residual moisture ($\theta$r). This occurred regardless of pineapple cultivar.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Aries Guineagrass (*Megathyrsus maximus* Jacq cv. Aries) pasture establishment without chemical control in an environmentally protected area

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**This study aimed to evaluate the Aries Guineagrass (*Megathyrsus maximus* sin. *Panicum maximum* Jacq. cv. Aries) and the weed community dynamics under different soil tillage and sowing methods in Southern Brazil, to create alternatives for sustainable farming in areas where weed chemical control is restricted. The experiment was carried out in split-plot design with randomized blocks and four replications; the main plots were three tillage methods: I) conventional, II) reduced tillage with moldboard plow, and III) reduced tillage with harrow plow; and the split-plots included two sowing methods: in line, (a) with seeds placed into the drill and (b) with seeds deposited on the soil surface. Aries Guineagrass and weeds were analyzed for phytosociological parameters during the pasture establishment. Lower weed densities and relative frequencies were found where the Aries Guineagrass was established under reduced tillage with moldboard plow and seeding on the soil surface. The Guineagrass establishment was effective as verified by the high plant survival rates (>75%) in the summer following the one that the pasture was sown. The results showed that, adequate soil tillage and sowing methods can further promote Aries Guineagrass development in areas where chemical control with herbicides is not allowed.**

**Key words:** Perennial pastures, phytosociology, tillage, weeds.

## INTRODUCTION

The current concern to achieve abundant and safe food production, with minimal impact on the environment, indicates that some sustainable agricultural practices, as conservation tillage with minimum soil disturbance, are essencial. In this way, in response to society demands, agricultural production systems must associate the increase in agricultural production with environmental quality (Lipper et al., 2014), thus promoting greater efficiency in the use of resources (Lemaire et al., 2014). Environmentally protected areas have been established by law to protect and conserve environmental resources or ecosystems, but they allow for human occupation, provided that this occurs in a sustainable and orderly manner. In a fair and non-exclusive way of the human component present, the knowledge of agricultural practices that attend to the activities that already occur in these areas is essential, since adaptations in the way of conducting these practices must be governed by sustainable principles and be pesticide-free.

The soil tillage method and crop sowing system can alter the seed bank dynamics (Palm et al., 2014; Schutte et al., 2014; Indoria et al., 2017) and, consequently, weed emergence. Soil tillage increase the concentration of seed of small plants at greater depths in the soil profile and reduce their germination (Vidal et al., 2007). Conventional preparation mixes the soil and weed seeds, concentrating soil aggregates larger than two centimeters near the soil surface, while weed seeds and finer soil particles are congregated at greater depths (Colbach et al., 2014).

In livestock systems, understanding how the weed community responds to soil tillage and forage management is an important conditioning to successful pasture development. These actions can alter the soil seed bank dynamics and provide a competitive forage species advantage in relation to weeds, mainly with no chemical control. The seed bank dynamics depends on environmental conditions and anthropic actions (Gardarin et al., 2012); also, agronomic practices should be understood as filters that determine the composition and abundance of functional characteristics of weeds in agroecosystems (Deiss et al., 2018).

In Brazil, the species *Panicum maximum* is considered one of the most widespread and important grass for livestock due to its high dry matter production, forage quality and ease of establishment (Oliveira et al., 2016). The Aries Guineagrass (*Megathyrsus maximus* sin. *P. maximum* Jacq. cv. Aries) is an F1 apomictic hybrid resulting from the artificial cross of *M. maximus* cv. Centauro with *M. maximus* cv. Aruana (Euclides et al., 2010). It is established by seeds, not affected by the severity of the cold and frost, typical of the winter climate in the Brazilian subtropical region.

The hypothesis of this work is that different soil preparation and sowing methods will alter the weed composition in the development of Aries guineagrass (*M. maximus* sin. *P. maximum* Jacq. cv. Aries) promoting a competitive advantage of the forage species in relation to weeds. Thus, alternative weed control practices can favor agricultural development in a sustainable way in environmentally protected areas where there are constraints to the use of agricultural pesticides. In this context, this study was evaluate to know how soil tillage and sowing methods affect the weed community and the establishment of Aries Guineagrass in environmentally protected areas of subtropical region of Brazil.

## MATERIALS AND METHODS

The experiment was conducted in Pinhais, State of Parana (25°24'S and 49°07'E, 900 m altitude), Brazil. It was located in the Rio Iraí Environmentally protected area, which is a territorial unit created by State Decree 1753/96 (PARANA, 1996), according to Law 6938/81 (BRASIL, 1981), which prohibits the use of herbicides, insecticides, acaricides, fungicides and nematicides in farming, but without restrictions as to the use of exotic species nor tillage and fertilizers.

The climate is humid subtropical, Cfb. The annual average rainfall is 1,400 to 1,600 mm and mean temperatures below 18°C in the coldest month and below 22°C in the warmer months (IAPAR, 2014). The soil is classified as Cambisol dystrophic tipic (USDA, 2014); the chemical profile of the soil collected at 20 cm depth showed the following values: pH ($CaCl_2$) = 5.1; P = 1.8 mg $dm^{-3}$; K = 0.16 cmolc $dm^{-3}$; Ca = 5.1 cmolc $dm^{-3}$; Mg = 3.6 cmolc $dm^{-3}$; Al = 0.0 cmolc $dm^{-3}$; H + Al = 4.8 cmolc $dm^{-3}$; SB = 8.86 cmol $dm^{-3}$; CTC = 13.6 cmolc $dm^{-3}$; V% = 64.5; and C = 31.8 g $dm^{-3}$.

The pasture species was the perennial summer Aries Guineagrass (*M. maximus* sin. *P. maximum* Jacq. cv. Aries). The soil was tilled on January 14, 2013 and forage was sown on January 17, 2013, with a density of 9 kg pelleted seeds per hectare, using base fertilization of 40 kg $ha^{-1}$ $P_2O_5$ (single superphosphate). Fifteen days after sowing, 300 kg $K_2O$ and 200 kg N in the form of potassium chloride and urea were applied, respectively. The survival of the Aries Guineagrass plants in the beginning of the next summer was evaluated by random marking of 108 plants, which were recounted in the month of September, 258 days after sowing, with the plants clumped.

The split plot design was performed with randomized blocks and four replications, totaling 24 experimental units. Soil preparation methods in the plots consisted of: 1) conventional tillage, consisting of tilling with a moldboard plow, followed by plowing with a harrow and a disc; 2) reduced tillage (chisel plow) with a moldboard plow, and 3) reduced tillage (chisel plow) with a harrow plow, and line forage sowing methods in the subplots by 1) seeds deposited in the soil within the sowing drill, and 2) seeds deposited on the soil surface. The plots had a 10 × 5 m size and were divided longitudinally for subplots allocation. In order to facilitate identification during the seedling phase, as well as to better evaluate the behavior of the forage species (Aries Guineagrass) in relation to other Poaceae, the "Poacea" group was created. This group included the species *Urochloa decumbens, Urochloa plantaginea*, *Cynodon dactylon* and *Digitaria horizontalis*.

For the plant community sampling, three 0.25 $m^2$ sites were randomly delimited in each experimental unit throughout the experimental period. Aries Guineagrass and weeds were properly identified and counted three times at 15, 33 and 58 days after forage sowing. The methodology used was proposed by Mueller-Dombois and Ellemberg (1974) and Braun-Blanquett (1979) to determine the total number of individuals (N), absolute frequency

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(AF), relative frequency (RF), absolute density (AD), relative density (RD), importance value index (IVI) and relative importance (RI). Data were submitted to analysis of the normality of residues as a conditioning factor to the analysis of variance (Anova). Duncan's test was used to compare values that were affected by significant factors and interaction (p <0.05). Statistica 8.0 software was used for the statistical analysis (Dell Software, 2007).

## RESULTS

Twenty-seven weed species distributed in fourteen families were identified (Table 1). *Euphorbia heterophylla*, *Raphanus raphanistrum* and Poaceae were the most relevant as to the botanical composition during Aries Guineagrass development, as they obtained higher frequency and relative density values (Table 2). The population with the highest relative density in the weed community belonged to the Poaceae group, followed by *E. heterophylla* and *R. raphanistrum*. On the other hand, the population showing the lowest relative density was *Richardia brasiliensis*, followed by *Sida rhombifolia* and *Ipomoea grandifolia*. Relative frequency indicated that plants from the Poaceae, *E. heterophylla, R. raphanistrum* and *Cyperus rotundus* groups were the most frequent species in all experimental area; *R. brasiliensis* showed the lowest frequency.

Soil preparation methods mobilized the seed bank dynamically, causing some weed species to show different densities and relative importance, while others were affected in their spatial distribution (i.e., frequency), as presented in Tables 3, 4 and 5.

The soil tillage management influenced ($P<0.05$) the relative density and importance of *E. heterophylla*, independently of sowing methods, only at 15 days after sowing. The sowing methods had no effect on the relative density and importance of *E. heterophylla* but they favored ($P<0.05$) the relative density and importance for Aries Guineagrass, regardless of soil preparation method (Table 3).

There was interaction ($P<0.05$) between soil tillage and sowing methods as for *R. raphanistrum* relative density and Poaceae relative frequency, represented by *U. decumbens*, *U. plantaginea* and *D. horizontalis* (Tables 4 and 5). *R. raphanistrum* density was not influenced ($P>0.05$) by soil tillage management when the Guineagrass was sown into the drill. Otherwise, the conventional tillage increased ($P<0.05$) this relative density if sowing was superficial (Table 4).

As shown in Table 5, when Aries Guineagrass was sown in the drill, the Poaceae relative frequency was higher ($P<0.05$) in conventional tillage as compared to reduced tillage with moldboard plow (Table 5). On the other hand, in-line surface sowing did not show differences ($P>0.05$) between the soil preparation methods.

Table 6 shows the absolute densities (plants.m$^2$) of Aries Guineagrass and the most relevant weed plants until 58 days after pasture establishment. As shown in Table 1, *R. raphanistrum, E. heterophylla* and Poaceae were the most relevant during Aries Guineagrass development, with evident decreasing of *E. heterophylla* at 58 days after sowing.

## DISCUSSION

According to the botanical composition (Table 1) at the Guineagrass establishment, it is possible to affirm that these plants are commonly present in the region. Kruchelski et al. (2019) studied the weed species in the establishment of Aries Guineagrass in same area and similar species (Table 2) were identified.

*R. raphanistrum* (wild radish) is an important weed (Cici and van Acker, 2009; Costa and Rizzardi, 2014) which has a large competition ability due to its largely viable seed production. According to Tricault et al. (2018), it is a highly competitive weed in winter crops.

Because of the importance of this weed, suitable methods of soil preparation and sowing may be an alternative to favor Aries Guineagrass development under unfavorable climatic conditions in environmentally protected areas that do not allow the use of chemical control for weeds. In this way, the conventional method, by promoting a larger number of weed seeds at greater depths (Mangin et al., 2018), may contribute to the establishment of Aries Guineagrass by reducing competition with weeds. From the 0.10 m depth, some weed seeds can germinate, however, they can become chlorotic, and consequently more susceptible to any method of management (Orzari et al., 2013).

Reduced tillage with a single harrow plowing favored the presence of *E. heterophylla* in relation to tillage with a moldboard plow; however, these two types of tillage did not affect the weed behavior when compared with conventional tillage. This fact is due to the deeper seed burial promoted by the moldboard plow tillage. Swanton et al. (2000) found a less uniform vertical distribution of weed seeds than the ones obtained by other methods of soil preparation, with most seeds buried at greater depths in the soil profile, 66% of which at 0.10 to 0.15 m.

Scheren et al. (2013) demonstrated that *E. heterophylla* shows a high emergence potential under satisfactory environmental conditions, which can damage the main crop, making it an important weed. *E. heterophylla* have great ability to absorb nitrogen, calcium and sulfur from the soil, which is intensified in the conventional method due to the greater mineralization of the organic matter provided by the soil rotation (Fontes and Morais, 2015). This has contributed to the higher relative density and frequency in conventional tillage.

Soil management by the use of a moldboard plow results in the presence of larger aggregates and increased macroporosity resulting from soil tillage, which reduces soil-seed contact thus reducing weeds' emergence (Colbach et al., 2014). In this way, the disc

**Table 1.** Weed species identified during the Aries Guineagrass (*Megathyrsus maximus* Jacq. cv. Aries) pasture establishment, Pinhais-PR, Brazil.

| Family | Scientific name | Common name | Life cycle |
|---|---|---|---|
| Asteraceae | *Bidens pilosa* | Hairy beggarsticks | Annual |
| Asteraceae | *Galinsoga parviflora* | Eclipta | Annual |
| Asteraceae | *Vernonia polysphaera* | Ironweed | Perennial |
| Asteraceae | *Xanthium strumarium* | Dandelion | Annual |
| Asteraceae | *Artemisia vulgaris* | Mugwort | Perennial |
| Brassicaceae | *Raphanus raphanistrum* | Wild radish | Annual |
| Brassicaceae | *Neslia paniculata* | Ball mustard | Annual |
| Cariophillaceae | *Silene gálica* | English catchfly | Annual |
| Convolvulaceae | *Ipomoea grandiflora* | Morningglory | Annual |
| Cyperaceae | *Cyperus rotundus* | Purple nutsedge | Perennial |
| Euphorbiaceae | *Euphorbia heterophylla* | Milkweed | Annual |
| Fabaceae | *Trifolium repens* | White clover | Perennial |
| Fabaceae | *Trifolium pratense* | Red clover | Biennial |
| Lamiaceae | *Stachys arvensis* | Fieldnelttle bentony | Annual |
| Malvaceae | *Sida rhombifolia* | Wire weed | Perennial |
| Oxalidaceae | *Oxalis oxiptera* | Woodsorrel | Annual |
| Papaveraceae | *Argemone mexicana* | Mexican prickle poppy | Perennial |
| Poaceae | *Avena strigosa* | Black oat | Annual |
| Poaceae | *Cynodon dactylon* | Bermuda grass | Perennial |
| Poaceae | *Urochloa decumbens* | Braquiária decumbens | Perennial |
| Poaceae | *Digitaria horizontalis* | Large cabgrass | Annual |
| Poaceae | *Urochloa plantaginea* | Alexander grass | Annual |
| Poaceae | *Sorghum halepense* | Jhonsongrass | Perennial |
| Poaceae | *Paspalum urvillei* | Vaseygrass | Perennial |
| Poaceae | *Paspalum paniculatum* | Capim-milhã | Perennial |
| Poligonaceae | *Rumex obtusifolius* | Curly dock | Perennial |
| Rubiaceae | *Richardia brasiliensis* | Brazil callality | Annual |

**Table 2.** Relative density, frequency and importance (%) of weed species, Aries Guineagrass (*Megathyrsus maximus* Jacq. cv. Aries) and the Poaceae family during the pasture establishment, Pinhais - PR, Brazil.

| Species | Relative density (%) | Relative frequency (%) | Relative importance (%) |
|---|---|---|---|
| *E. heterophylla* | 15.7 | 10.3 | 13.0 |
| *R. raphanistrum* | 13.5 | 10.1 | 11.8 |
| *B. pilosa* | 9.3 | 9.7 | 9.5 |
| *S. rhombifolia* | 1.5 | 4.9 | 3.2 |
| *G. parviflora* | 7.8 | 9.4 | 8.6 |
| *I. grandiflora* | 6.1 | 9.5 | 7.8 |
| *R. brasiliensis* | 0.9 | 3.6 | 2.3 |
| *C. rotundus* | 9.7 | 10.1 | 9.9 |
| Aries Guineagrass Aries | 9.6 | 9.3 | 9.5 |
| Poaceae* | 19.7 | 10.3 | 15.0 |

*Poaceae (*Urochloa decumbens, U. plantaginea, Cynodon dactylon* and *Digitaria horizontalis*).

plow may have contributed to maintain the *E. heterophylla* seed bank closer to the soil surface in relation to the moldboard plow, thus intensifying its potential interference ability on Aries Guineagrass. According to Gruber et al. (2012), the disc plow, mobilizes the soil less intensely than conventional tillage

**Table 3.** Relative density (%) and relative importance (%) of *Euphorbia heterophylla* and Aries Guineagrass (*Megathyrsus maximus* Jacq. cv. Aries), 15 days after sowing, under different soil preparation and sowing methods, Pinhais - PR, Brazil.

| Species | Soil preparation methods | | | Sowing methods | |
|---|---|---|---|---|---|
| | Conventional tillage | Reduced tillage + moldboard plow | Reduced tillage + harrow plow | Into the drill | On soil surface |
| | | | Relative density (%) | | |
| *E. heterophylla* | $19.25^{ab}$ | $15.38^{b}$ | $24.77^{a}$ | 21.82 | 17.78 |
| Aries Guineagrass | 10.30 | 12.55 | 12.23 | $7.97^{B}$ | $15.42^{A}$ |
| | | | Relative importance (%) | | |
| *E. heterophylla* | $15.43^{ab}$ | $13.19^{b}$ | $18.11^{a}$ | 16.80 | 14.36 |
| Aries Guineagrass | 10.10 | 11.77 | 10.69 | $8.54^{B}$ | $13.17^{A}$ |

Within each species, different uppercase letters between soil preparation methods and lowercase letters between sowing methods indicate difference by the Duncan test ($p < 0.05$).

**Table 4.** Relative density (%) of *Raphanus raphanistrum* under different soil preparation and sowing methods of Guineagrass cv. Aries (*Megathyrsus maximus* Jacq. cv. Aries), Pinhais - PR, Brazil.

| Soil preparation methods | Sowing methods | |
|---|---|---|
| | Into the drill | On soil surface |
| | Relative density (%) | |
| Conventional tillage | $12.56^{Ab}$ | $20.28^{Aa}$ |
| Reduced tillage + moldboard plow | $15.73^{Aa}$ | $9.57^{Bb}$ |
| Reduced tillage + harrow plow | $11.68^{Aa}$ | $11.26^{Ba}$ |

Different uppercase letters between soil preparation methods and lowercase letters between sowing methods indicate difference by the Duncan test ($p < 0.05$).

**Table 5.** Relative frequency of the Poaceae family (*Urochloa decumbens, U. plantaginea, Cynodon dactylon* and *Digitaria horizontalis*) under different soil preparation and sowing methods of Guineagrass cv. Aries (*Megathyrsus maximus* cv. Aries), Pinhais - PR, Brazil.

| Soil preparation methods | Sowing methods | |
|---|---|---|
| | Into the drill | On soil surface |
| | Relative frequency (%) | |
| Conventional tillage | $11.02^{Aa}$ | $10.08^{Ab}$ |
| Reduced tillage + moldboard plow | $9.76^{Ba}$ | $10.36^{Aa}$ |
| Reduced tillage + harrow plow | $10.50^{ABa}$ | $10.06^{Aa}$ |

Different uppercase letters between soil preparation methods and lowercase letters between sowing methods indicate difference by the Duncan test ($p < 0.05$).

and the use of the moldboard plow exclusively.

Surface in-line seeding showed higher values of relative importance for Aries Guineagrass as compared to drill sowing. This may have occurred because the seeds remained on the surface, thus favoring their development, as opposed to the seeds deposited in the drill, which may have been buried at greater depths in the soil profile during the disaggregation and accommodation of soil clods after sowing, and therefore had delayed plant emergence.

Thus, *R. raphanistrum* seeds move to greater depths may have prevented their emergence, either due to the environmental conditions that may have inhibited their germination, such as variations in temperature and light (Copeland and McDonald, 2001) or because of the depletion of reserves by the plants that germinated but did not emerge to begin their growth. The high absolute density of *R raphanistrum* at 15 days after sowing (Table

**Table 6.** Absolute density (plants.m$^{-2}$) of *Megathyrsus maximus* Jacq. cv. Aries, weeds and the Poaceae family at 15, 33 and 58 days after sowing, Pinhais - PR, Brazil.

| **Species** | **Days after sowing** | | |
|---|---|---|---|
| | **15** | **33** | **58** |
| *Megathyrsus maximus* cv. Aries | 9.6 | 9.7 | 7.1 |
| *Euphorbia heterophylla* | 16.1 | 15.2 | 11.3 |
| *Raphanus raphanistrum* | 18.6 | 12.9 | 6.5 |
| *Bidens pilosa* | 5.3 | 13.1 | 8.6 |
| *Sida rhombifolia* | 0.4 | 2.1 | 1.8 |
| *Galinsoga parviflora* | 3.5 | 10.3 | 8.3 |
| *Ipomoea grandiflora* | 4.4 | 5.8 | 6.4 |
| *Richardia brasiliensis* | 0.0 | 2.1 | 0.9 |
| *Cyperus rotundus* | 5.7 | 10.7 | 9.6 |
| Poaceae* | 13.5 | 19.7 | 21.2 |

*Poaceae (*Urochloa decumbens, U. plantaginea, Cynodon dactylon* and *Digitaria horizontalis*).

6) may have triggered, over time, allelopathic action on the surrounding weeds, similarly to the behavior observed in the southeastern region of the United States (Norsworthy, 2003), contributing to the reduction of the absolute density values of most of the weeds.

The greater soil mobilization in the conventional tillage with sowing in the drill provided greater Poaceae occurrence in the plant community. Favreto and Medeiros (2006) also found high presence of Poacea *U. plantaginea* in conventional tillage compared to the direct sowing in range pasture area in the Central Depression of Rio Grande do Sul, Brazil. With the superficial sowing of the Guineagrass, the relative frequency of the Poaceae family (*U. decumbens, U. plantaginea, C. dactylon* and *D. horizontalis*) was not influenced by the soil preparation methods, probably because there was no opening of sowing drill and the exposure of seeds to environment was similar between the different tillage methods.

The Aries Guineagrass absolute density with no use of herbicides for weed chemical control was similar to that observed by other authors under conventional management (with chemical weed control). Gerdes et al. (2002) reported 9.0, 11.0 and 10.0 plants m$^{-2}$ (absolute density, AD) for Guineagrass cv. Tanzania at 7, 14 and 21 days after sowing under soil tillage conditions and use of desiccant herbicide for the elimination of *U. decumbens* and other weeds. This points out to a possibility of success in the development of Aries Guineagrass without the use of herbicides.

The adoption of competitive crops, changes in sowing dates and soil fertilization are cultural practices that must be implemented in conservation agriculture (Nichols et al., 2015). In this sense, the survival of Aries Guineagrass was 75%, estimated for 258 days after sowing, with a standard error deviation of 4.2. This highlights the ability of Aries Guineagrass to survive even under weed competition conditions. Because it is an exotic summer perennial species of C4 metabolism, developing in late summer and early fall, Aries Guineagrass had to compete with weeds adapted to the climatic conditions at this time of year, which are less favorable to the development of this group of forage species, and more favorable to the group of weeds studied, as we have emphasized for *R. raphanistrum* weed. However, Aries Guineagrass development was effective, which demonstrated the viability of the management practice used.

In addition to this, the pasture was sown relatively late when compared with the common sowing periods for tropical grasses in that region (the middle of summer vs. spring, respectively). Late sowing of this species may be a disadvantage for pasture development, which may be due to the high competitiveness of the weed community that settles at the beginning of the hot season, especially in areas where no chemical control is allowed. In addition, in subtropical and temperate regions, the growing season is followed by lower temperatures of fall, which are associated with the slow development of the grass and the rapid growth of other weed species, more adapted to these environmental conditions.

## Conclusion

From the results of the present study, it was concluded that the conventional tillage, reduced with moldboard plow as reduced with disc plow methods, allows Aries Guineagrass development, especially in the case of in-line sowing on the soil surface. Reduced soil tillage with single moldboard plow allows better weed management and greater competitive advantage of Aries Guineagrass for pasture establishment. The use of adequate soil tillage and sowing methods is an alternative to benefit Aries Guineagrass development in environmentally protected areas where the chemical control with herbicides is not allowed.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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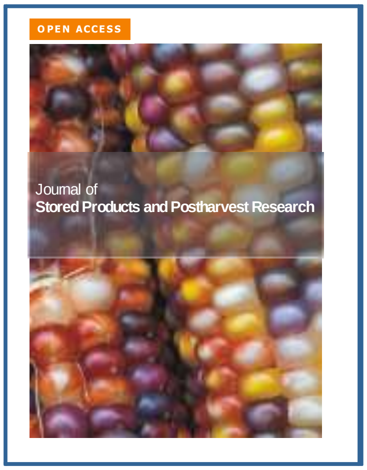
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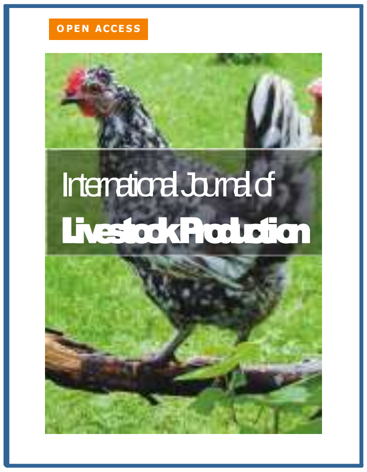
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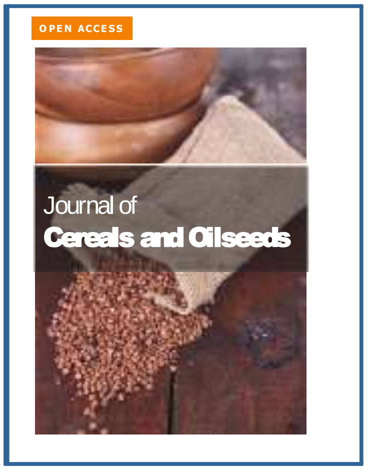
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