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Stalk and Root Development of Sugarcane Treated with Nitrogen, Filter Cake and Inoculation of Diazotrophs

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Abstract: In order to gain better insight into the development of shoots, a more comprehensive understanding of the behavior of the root system is needed. The aim of this study was to analyze the response of the roots, the plant stalk and ratoon cane for the cultivars RB867515 and RB92579 after treatment with diazotrophic bacteria, either alone or in conjunction with the filter cake and nitrogen. The study was conducted in rhizotron condition at the Sugarcane Research Station (SCA-UFPR). Assessments of shoots and roots were performed at 60, 120, 180, 240, 300 and 360 days after planting. The length of the root system during the cycle was evaluated by the tracing method. At the end of the cycle, the roots were collected using a volumetric cylinder and evaluated with image analyzer which defined the length, surface area, volume and root diameter. During the cycle, the diameter, height, number of stalks and leaf area were measured. At the end, the stalks were evaluated by determining their weight. It was also quantified the foliar nitrogen and isolated the nitrogen fixing bacteria. It was observed that the diazotrophic bacteria did not alter the agronomic characteristics of the root system or the technological quality. The filter cake allowed greater root growth, especially in the initial phase of the cycle. This may have resulted from the retention of a greater number of stalks, resulting in higher tons of cane per hectare (TCH) and tons of pol per hectare (TPH). It was possible to observe a significant correlation of the length and volume of the root system with TCH and TCH with the number and height of the stalks. The tracing method used to estimate the decal root length was effective when compared to the image analysis method.

Key words: Root, nitrogen-fixing bacteria, *Saccharum* spp.

INTRODUCTION

To fully understand the phenomena that occur in plant shoots, it is necessary to have a complete understanding of what happens below the surface of the soil, especially in relation to the growth and distribution of roots (Vasconcelos *et al*., 2003). According to Vasconcelos and Garcia (2005), the extension, distribution and activity of the roots determine the amount of water and nutrients that are absorbed which are essential for the development of the crops.

Compared to other methods, using the rhizotron to study root systems has the advantage of allowing for the analysis of the distribution and length of the root system at different time intervals for the same plant without its destruction. However, there are only limited studies using this system for sugarcane (Ido *et al*., 2006).

Inoculation with diazotrophic bacteria may allow culture benefits, as previously reported (Marques *et al*., 2008; De Silva *et al*., 2009; Skolaude *et al*., 2010). The major bacteria are the *Gluconacetobacter*, *Herbaspirillum* and *Azospirillum* (Dobereiner, 1992), stands out as one of the most promising *Gluconacetobacter diazotrophicus* (Barbosa *et al*., 2006). Thus, it can be an alternative to replace the nitrogen.

In relation to nitrogen fertilization, it is essential to have a better knowledge of this practice, since the mismanagement of a cane field, especially with nitrogenous fertilizer, nitrogen can result in either reduced crop productivity or their longevity (Vitti *et al*., 2007). Furthermore, in maturing, a by-product that has been highlighted is the filter cake, it has widely varied nutrient composition values, typically showing reasonable levels of nitrogen and phosphorus and relatively low levels of potassium (Pereira *et al*., 2003). It could be an option for increasing productivity upto 20 Mg ha^{-1} (Dinardo-Miranda *et al*., 2003), promoting the improvement of soil fertility (De Almeida *et al*., 2011), without changing its technological characteristics (Santos *et al*., 2010).

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Because of this, it is important to better understand the behavior of the root cultivars RB867515 and RB92579, since they are widely cultivated in Brazil, with areas of 1,311 and 372,000 ha, respectively which corresponds to 22.1 and 6.3% of acreage, respectively (RIDESA, 2011).

The aim of this study was to analyze the response of the roots, plant stalk and ratoon cane for the cultivars RB867515 and RB93579 after treatment with diazotrophic bacteria, either alone or in conjunction with a filter cake and nitrogen.

MATERIALS AND METHODS

This study was carried out at the Sugarcane Research Station, in Paranavaí-PR which belongs to Federal University of Paraná. Planting was done on November 5, 2010, the plant cane was harvested on November 13, 2011 and ratoon cane was harvested on November 7, 2012. The studied cultivars were RB867515 and RB92579. The soil used in the experiment was collected at the Experimental Station and classified a Ferralsol (Dystric) (FAO, 2006) (Table 1).

The experiment was conducted in a structure called Rhizotron that has a trapezoid shape and has internal sheets of transparent glass which allow to observe and analyze the development of the root system. And externally, the structure has sheets of amianthus lined with styrofoam, avoiding the sun heating these sheets and interfering in the growth of the root system.

The dimensions of the Rhizotron were 2.05 m tall, 7 m long, with a base that is 4.22 m wide and a top that is 2.20 m, resulting in a slope of 25°. This slope favored the growth of representative parts of the root system near the glass, allowing visualization. The compartments where the plants grew were 0.25 m wide, 1 m long and 2.05 m tall.

The treatments conditions were: (a) T1 is non-inoculated, (b) T2 is inoculated, (c) T3 is non-inoculated and 80 kg N ha^{-1}, (d) T4 is inoculated and 80 kg N ha^{-1} N, (e) T5 is non-inoculated and 14 Mg filter cake ha^{-1} and (f) T6 is inoculated and 14 Mg filter cake ha^{-1}. These treatments were repeated after harvesting the plant cane. Irrigation drip system was used to keep the soil close to the field capacity.

The fertilizer used in all treatments consisted of the equivalent of 140 kg P_2O_5 ha^{-1} and 160 kg K_2O ha^{-1}. For plants treated with nitrogen, an amount equivalent to 80 kg N ha^{-1} was used. The fertilizer was positioned approximately 10 cm below the 1-bud sett. For the treatments using the filter cakes, an amount equivalent to 14 Mg N ha^{-1} was used along with the mineral fertilizer.

The inoculated treatments consisted of inoculation (peat inoculant) with a mixture of 5 bacteria (strains) provided by Embrapa Agrobiologia: *Gluconacetobacter diazotrophicus* (PAL5), *Herbaspirillum seropedicae* (HRC54), *Herbaspirillum rubrisubalbicans* (HCc103), *Azospirillum amazonense* (CBAmC) and *Burkholderia tropica* (Ppe8). It was used as 75 g dissolved in 14 L of water. The stalk cuttings (all of 1-bud sett) were immersed in this solution for 30 min and then immediately planted.

In order to perform the foliar nitrogen analysis, the leaves (leaf+1) were collected according to procedures described by EMBRAPA (2005) and subsequently analyzed according to the methodology described by Tedesco *et al*. (1995). The method of growth analysis and distribution of the root system was made by the decal (tracing) method, where 5 evaluations were performed at 60, 120, 180, 240 and 300 Days After Planting (DAP). The decal method involves superimposing a plastic slide over the rhizotron glass and copy roots that are visible using a stylus of different colours at each evaluation to facilitate subsequent reading with a map measurer. Thus, it was possible to quantify the length and concentration of the root system in each extract during each assessment (COMPD).

During the final evaluation, samples were collected after the removal of the sheets of amianthus of each compartment at depths of 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 cm. A 304 cm^3 soil volume was used with a volumetric cylinder. Subsequently, the samples were placed in plastic containers and then washed with running water with sieves. Later, after all the curious materials removed, the roots were stored in plastic pots containing alcohol (50%). Root samples were evaluated using the Scanner LA1600 and the WinRhizo computer program to quantify the following variables: Length (cm) (COMPW), specific surface area (cm^2), diameter (mm) and root volume (cm^3).

The variables analyzed were (1) Green mass of stalks (kg), (2) No. of stalks, (3) No. of green leaves which were included from leaf 0 and had at least 20% of green area, (4) Plant height (cm), the substrate level up to the

Table 1: Results of chemical and physical analysis of the soil, Analysis was performed at the Laboratory of Soils of the Soils Department at the Federal University of Parana

	Chemistry ($cmol_c$ dm^{-3})										Physics (g kg^{-1})		
pH $CaCl_2$	Al^{+3}	H^+Al^{+3}	Ca^{+2}	Mg^{+2}	K^+	T_{CEC}	CEC	P (mg dm^{-3})	C (g dm^{-3})	BS (%)	Sand	Silt	Clay
5.60	0	1.18	1.4	0.8	0.04	2.24	4.04	12	7.8	55	841	9	150

CEC: Cation exchange capacity = H+Al+Ca+Mg+K, Total CEC: T_{CEC} = Ca+Mg+K, BS: Base saturation = (T_{CEC}/CEC)×100

leaf +1, (5) Diameter (cm), measured at the first internode and (6) Leaf area (cm^3) of all tillers, estimated by the equation proposed by Hermann and Camara (1999):

$$AFc = C \times L \times 0.75 \times (N+2)$$

where, AFc is leaf area, C is +3 leaf length, L is largest width of the leaf +3; 0.75 is shape factor; N is No. of leaves completely open and with at least 20% of green area (leaf 0 to +7) and 2 is correction factor. Thereafter, the sum of all leaf areas of each tiller to obtain the sum total of each treatment was carried out.

The cane yield (tons of cane per hectare) was calculated according to the methodology proposed by Landell (1995) by the following equation:

$$TCH = (d^2 \times C \times h \times 0.007854)/E$$

where, 'd' is the average diameter of the stalks (cm), 'C' is No. of tillers m^{-1}, 'h' is the average height of the stalks (cm) and 'E' is the spacing between compartments which was 1 m in this case. The density of stalks was considered equal to one.

We also analyzed the technological characteristics: Pol, fiber, total recoverable sugar (ATR) and tons of pol per hectare (TPH or yield).

The results were submitted for analysis of the variance and the means were compared by the Tukey test at 5% of probability using the software system for analysis of variance (SISVAR). And the correlations (Pearson) were generated by the program Sigmaplot 11.0.

RESULTS AND DISCUSSION

Figure 1 and 2 show the average behavior of the plant cane and first ratoon cane related by the root system and TCH (yield or tons of cane ha^{-1}) for the cultivars RB92579 and RB867515 during the crop cycle based on the treatments used.

At 60 DAP, during the first evaluation of cultivars RB92579, it was possible to observe that the longest root system for the filter cake treatments. The root lengths for treatments A, B, C, D, E and F were 2309, 2021, 2349, 1666, 3966 and 3494 cm $plant^{-1}$, respectively. These values decreased throughout the crop cycle, showing root lengths of 812, 950, 855, 840, 835 and 947 cm $plant^{-1}$, respectively, at 300 DAP.

The yield observed at 60 DAP was 33.0, 30.5, 45.9, 55.9, 71.6 and 54.5 Mg ha^{-1} for treatments A, B, C, D, E and F, respectively. The yield at 300 DAP was 168, 182, 220, 182, 290 and 267 Mg ha^{-1} for treatments A, B, C, D, E and F, respectively.

Similar results were obtained for the cultivar RB867515 and during the first assessment (60 DAP). For treatments with filter cake (E and F), there was greater initial length of the root system. The length of roots of treatments A, B, C, D, E and F, respectively are 2246, 1987, 2820, 2103, 3965 and 3493 cm, decreasing throughout the cycle, reaching values of 300 DAP 416, 620, 731, 612, 880 and 839 cm. The final yield observed were 182, 172, 191, 190, 252 and 261 Mg ha^{-1} for treatments A, B, C, D, E and F, respectively. The largest growth of the root system may have resulted in higher yields.

The greater root growth for the filter cake-treated samples may be due to the higher moisture content, as suggested by Duruoha *et al.* (2001), since the roots respond well to the presence of moisture in the soil and the filter cake provides a greater capacity to retain water in the soil (Penatti and Donzelli, 1991). Ido *et al.* (2006) also using rhizotron condition, observed that the substrate affects the growth of the root system. The variables for the root system are presented in Table 2 to RB92579 and Table 3 to RB867515. It can be seen that root length was superior to treatment in which inoculation plus further application of filter cake, followed by the treatment with a filter cake, by either the method of volumetric cylinder or by the decal method.

Table 2: Evaluation of both harvest (plant cane and first ratoon) of length (COMPW), surface area (SA), diameter (DIA) and volume (VOL) as determined by WinRhizo and root length determined by the decal method (COMPD) for the diffrent treatments of cultivar RB92579

Treatments	COMPD (cm)	COMPW (cm)	AS (cm^2)	VOL (cm^2)
T1	1375^{c}	4531^{d}	173.5^{b}	42.2^{bc}
T2	1377^{c}	5078^{c}	156.8^{b}	41.0^{bc}
T3	1698^{b}	5502^{b}	168.6^{b}	40.9^{bc}
T4	1441^{c}	4307^{d}	121.6^{c}	38.1^{c}
T5	1958^{a}	6678^{a}	205.9^{a}	53.0^{a}
T6	1772^{b}	5724^{b}	179.1^{b}	49.7^{ab}

Means followed by the same letter in the column do not differ by the Tukey test at 5%, T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

Table 3: Evaluation of both harvest (plant cane and first ratoon) of length (COMPW), surface area (SA), diameter (DIA) and volume (VOL) as determined by WinRhizo and root length determined by the decal method (COMPD) for the diffrent treatments of cultivar RB867515

Treatments	COMPD (cm)	COMPW (cm)	AS (cm^2)	VOL (cm^2)
T1	1205^{c}	4781^{b}	146.5^{b}	37.0^{b}
T2	1318^{c}	5252^{ab}	167.8^{b}	41.4^{b}
T3	1426^{bc}	4926^{b}	150.1^{b}	39.9^{b}
T4	1536^{abc}	5675^{ab}	198.6^{a}	43.7^{ab}
T5	1714^{ab}	5862^{ab}	187.7^{a}	51.4^{a}
T6	1783^{a}	6460^{a}	198.2^{a}	49.5^{a}

Means followed by the same letter in the column do not differ by the Tukey test at 5%, T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

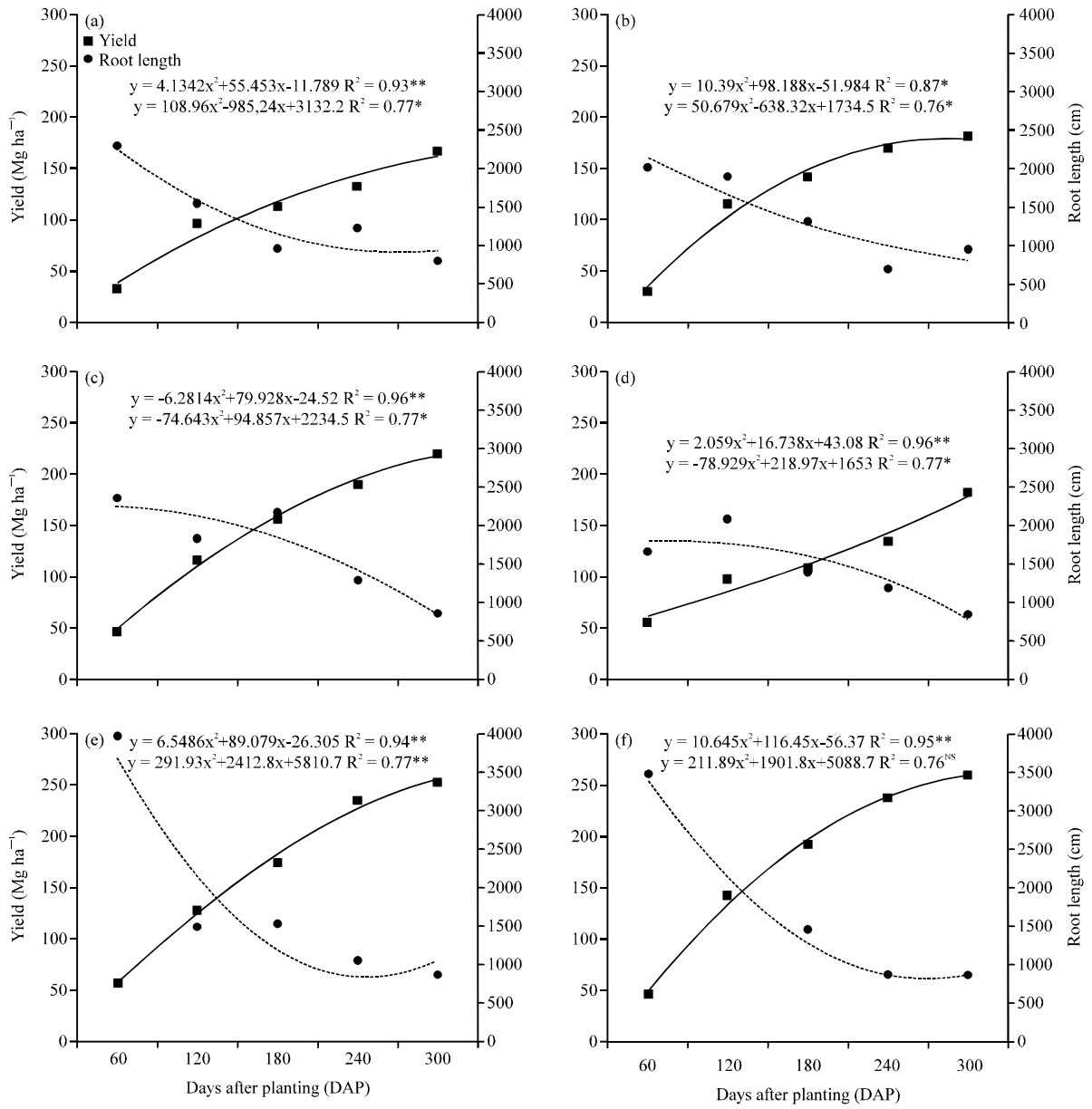

Fig. 1(a-f): Average behavior (plant cane and first ratoon cane) of root length (cm) and tons of cane ha^{-1} (TCH-yield), to the treatments (a) Non-inoculated (b) Inoculated, (c) Non-inoculated and 80 kg N ha^{-1}, (d) Inoculated and 80 kg N ha^{-1}, (e) Non-inoculated and 14 Mg filter cake ha^{-1} and (f) Inoculated and 14 Mg filter cake ha^{-1} at 60, 120, 180, 240 and 300 DAP for the cultivar RB92579

It can be checked in Table 2 and 3, similar values to the other variables. Root surface area and root volume, for both cultivars, were statically superior in treatments with a filter cake. The worst treatments were non-inoculated and inoculated ones (T1 and T2).

For the variables of the shoot for the cultivar RB92579 (Table 4), the treatments that included the filter cake gave the greatest yield, differing significantly from the other treatment conditions. The values reached 299 and 271 Mg ha^{-1} when used the filter cake either without (T5) and with inoculation (T6), respectively. This higher yield was associated with the highest production of stalk per clump which were 15.5 and 14 for the treatment conditions when the filter cake was used (T5 and T6). These results also differed from the other treatments.

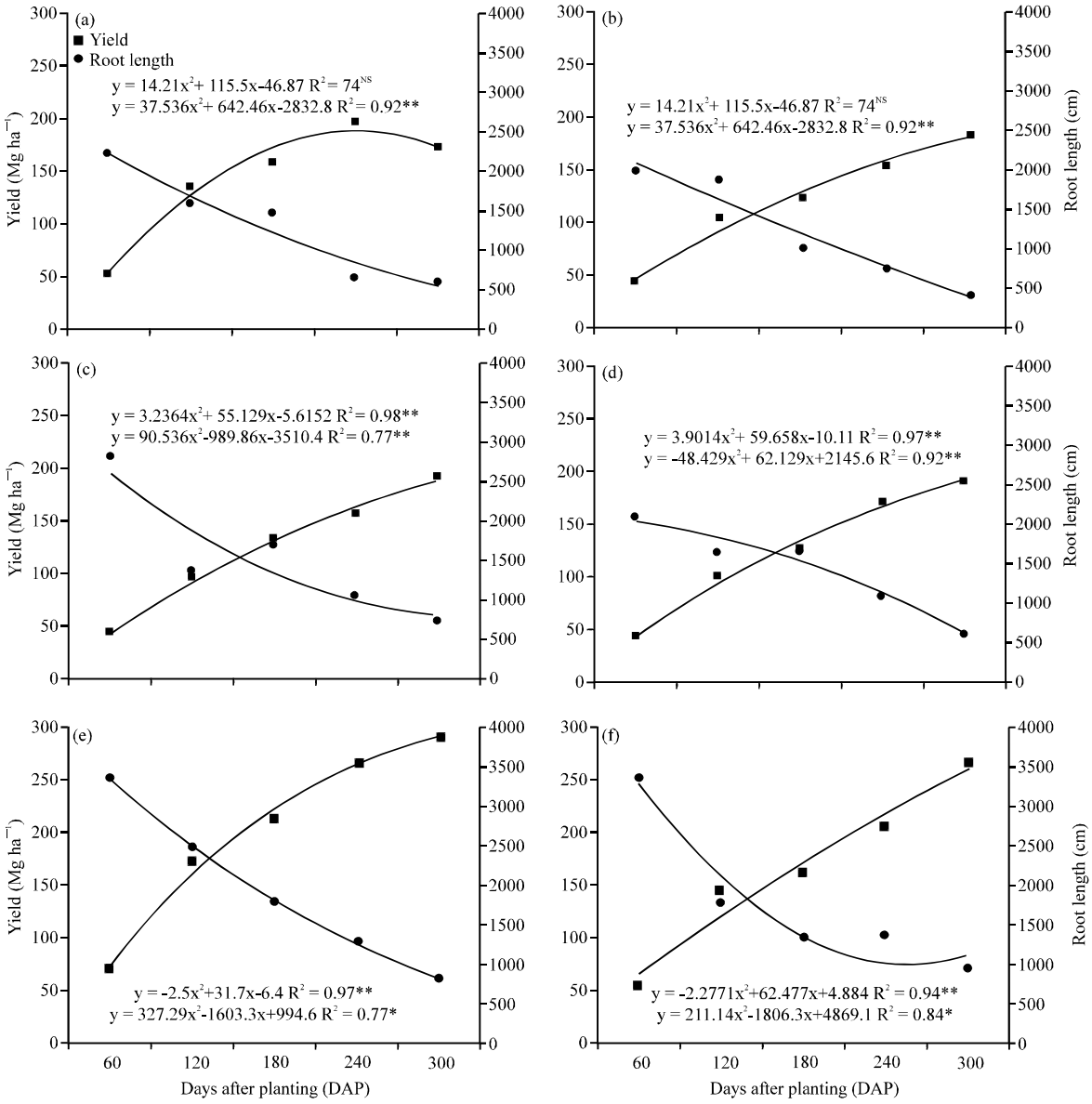

Fig. 2(a-f): Average behavior (plant cane and first ratoon cane) of root length (cm) and tons of cane h^{-1} (TCH-yield), to the treatments (a) Non-inoculated, (b) Inoculated, (c) Non-inoculated and 80 kg N ha^{-1}, (d) Inoculated and 80 kg N ha^{-1}, (e) Non-inoculated and 14 Mg filter cake ha^{-1} and (f) Inoculated and 14 Mg filter cake ha^{-1} at 60, 120, 180, 240 and 300 DAP for the cultivar RB867515

Table 4: Evaluation of both harvest (plant cane and first ratoon) for agronomic traits of cultivar RB92579

Treatments	NC	EST (cm)	DIA (mm)	AF (cm^2)	M1C (kg)	TCH (Mg ha^{-1})
T1	10.0^{b}	232.0^{c}	2.93^{a}	3338^{a}	1.11^{a}	160^{d}
T2	9.5^{b}	233.1^{c}	3.24^{a}	3642^{a}	1.34^{a}	182^{cd}
T3	10.0^{b}	261.7^{bc}	3.15^{a}	3949^{a}	1.45^{a}	225^{b}
T4	10.0^{b}	239.0^{bc}	3.20^{a}	3218^{a}	1.41^{a}	193^{c}
T5	15.5^{a}	266.4^{a}	3.04^{a}	3614^{a}	1.36^{a}	299^{a}
T6	14.0^{a}	259.8^{bc}	3.08^{a}	3739^{a}	1.38^{a}	271^{a}

Means followed by the same letter in the column do not differ by the Tukey test at 5%, NC: No. of stalks per compartment, EST: Mean height of plants, DIA: Average diameter, AF: Leaf area $plant^{-1}$, MIC: Mass of a stalk and TCH: Cane yield for the treatments, T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

Table 5: Evaluation of both harvest (plant cane and first ratoon) for agronomic traits of cultivar RB867515

Treatments	NC	EST (cm)	DIA (mm)	AF (cm^2)	M1C (kg)	TCH (Mg ha^{-1})
T1	8.0^{b}	244.3^{bc}	3.4^{a}	3577^{a}	1.54^{a}	183^{c}
T2	9.0^{ab}	216.8^{c}	3.3^{a}	2808^{a}	1.41^{a}	169^{c}
T3	9.0^{ab}	263.1^{b}	3.4^{a}	3638^{a}	1.69^{a}	227^{b}
T4	9.0^{ab}	240.9^{bc}	3.2^{a}	2799^{a}	1.45^{a}	189^{c}
T5	10.0^{a}	296.0^{a}	3.3^{a}	3932^{a}	1.82^{a}	264^{a}
T6	12.5^{a}	260.9^{b}	3.2^{a}	3516^{a}	1.42^{a}	265^{a}

Means followed by the same letter in the column do not differ by Tukey test at 5%,NC: No. of stalks per compartment, EST: Mean height of plants, DIA: Average diameter, AF: Leaf area per plant, MIC: Mass of a stalk and TCH: Cane yield for the treatments, T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

Additionally, the treatment without inoculation and including the filter cake (T5) resulted in an average plant height of 266.4 cm, better than other treatments. However, no significant differences were observed when it was used inoculation with filter cake, nitrogen without inoculation and inoculation with nitrogen. For the diameter, leaf area and the mass of the stalk, there were non-significant differences.

Similar results were obtained for the cultivar RB867515 (Table 5), where treatments that included the filter cake resulted in greatest yield, differing significantly from other treatments. This highest production of yield was associated with the largest number of stalks: 10 and 12.5 stalk for treatments without and with inoculation in addition to the filter cake, respectively. The average height in the non-inoculated treatment and with a filter cake had the highest value and differed from the others. For the diameter, leaf area and the mass of a stalk, there were not significantly different within the treatments.

It was demonstrated that the root system may have contributed positively to the biomass, as has been described by Rocha *et al.* (2008). Probably, the root system allows the plant cane deep exploration of the soil and the best use of water and nutrients, contributing to development of shoots.

The influence of the treatments on the technological characteristics of pol, fiber, ATR and TPH for the cultivar RB92579 can be observed in Table 6. It was observed that the treatment non-inoculated further application of filter cake (T5) resulted the highest TPH (45.2 Mg ha^{-1}), differing significantly from the other treatments. The higher yield was attributed to highest values obtained of yield in T5, because pol was not statistically different within the treatments.

For the cultivar RB867515 (Table 7), it was observed for TPH, the treatments with a filter cake giving the best values. However, differently of results in RB92579, the treatments with nitrogen fertilizer (T3 and T4) showed greater values for pol. The treatment without a filter cake, nitrogen and inoculation was the worst one.

It was possible to detect small decreases in pol caused by the filter cake, although this difference was not statistically significant. This was also reported by Penatti and Boni (1989) when increasing doses of filter cake (0, 3, 6 and 9 Mg ha^{-1}) were applied in the furrow. The author believed that this can be explained by the higher moisture accumulation in these treatments which makes it difficult to accumulate sucrose. However, Santos *et al.* (2010) using dose combinations of filter cake and soluble phosphate, observed no change in the quality of sugarcane juice when evaluating the brix of the juice.

Subsequently, Santos *et al.* (2011) found that phosphorus with filter cake applied at planting improved the quality of the sugarcane by increasing the content of soluble solids (°Brix) and reducing total sugars (ART) and sucrose in stalks (TPH). Silva *et al.* (2010) when using a filter cake (30 Mg ha^{-1}) alone and in combination with a conventional fertilizer (500 kg ha^{-1}) also found no significant effects on the ATR, pol and °Brix.

The results of the effects of inoculation on the foliar nitrogen levels in plant cane and first ratoon allowed us to determine that there was no benefit from the use of

Table 6: Evaluation of both harvest (plant cane and first ratoon) of the technological characteristics of pol, fiber, ATR and TPH for the diffrent treatment conditions of cultivar RB92579

Treatments	Pol (%)	Fiber (%)	ATR (kg mg^{-1})	TPH (Mg ha^{-1})
T1	15.5^{a}	14.0^{a}	148.3^{a}	25.6^{e}
T2	16.0^{a}	13.7^{a}	152.1^{a}	29.0^{d}
T3	15.3^{a}	13.7^{a}	144.6^{a}	34.7^{c}
T4	16.4^{a}	13.6^{a}	155.3^{a}	30.1^{d}
T5	15.3^{a}	12.7^{a}	150.6^{a}	45.2^{a}
T6	15.8^{a}	13.2^{a}	156.1^{a}	42.6^{b}

Means followed by the same letter in the column do not differ by the Tukey test at 5%,T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

Table 7: Evaluation of both harvest (plant cane and first ratoon) of the technological characteristics of pol, fiber, ATR and TPH for the diffrent treatment conditions of the cultivar RB92579

Treatments	Pol	Fiber	ATR (kg mg^{-1})	TPH (Mg ha^{-1})
T1	14.0^{b}	14.5^{a}	136.6^{a}	25.4^{c}
T2	15.5^{ab}	13.4^{a}	151.0^{a}	27.4^{bc}
T3	17.2^{a}	14.1^{a}	164.1^{a}	32.7^{b}
T4	16.3^{a}	13.4^{a}	160.5^{a}	31.4^{b}
T5	15.9^{ab}	14.8^{a}	156.5^{a}	41.6^{a}
T6	15.3^{ab}	14.7^{a}	151.0^{a}	38.5^{a}

Means followed by the same letter in the column do not differ by the Tukey test at 5 %, T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

nitrogen fixing bacteria for nitrogen leaf accumulation. Likewise, the benefits of inoculation in other aspects cannot be identified in this work as demonstrated in Table 8.

The population of bacteria *Burkholderia tropica* isolated from the cultivar RB92579 demonstrated the highest population in the inoculated treatments (T2, T4 and T6) (Table 9). For the cultivar RB867515 when inoculated alone with diazotrophic bacteria (T2) was the same population of treatment non-inoculated with a filter cake (T5) (Table 10). *Gluconacetobacter diazotrophicus* also was the greatest population in inoculated treatments (T2, T4 and T6) of RB92579 (Table 9) and was not different in RB867515 (Table 10). The bacteria *Azospirillum amazonense* had the similar results of *G. diazotrophicus* in the inoculated treatments in both cultivars (Table 9 and 10). The genus *Herbaspirillum* spp. was not different in both cultivars.

For the root shoots, using both the decal (CRD) method and by the method of image analyser (CRW), the root length was significant with TCH (r = 0.81 and 0.60). TCH had significant correlation with the number of stalk (r = 0.68) and height (r = 0.84), but not with the stalk diameter (r = 0.21).

Santana *et al.* (2010) observed that the average diameter of stalk showed highly significant and positive correlation with the average weight of the stalk, but it did not show a significant correlation with an average height of stalk and leaf area average. However, the average weight of the stalk was correlated significantly and positively with the average height of stalk and leaf area average.

Regarding the correlations performed in the root system, it can be concluded that the methodology of evaluation of root length by the decal method correlated positively and significantly with the cylinder method or volumetric image analysis (r = 0.86) and thus one efficient method to estimate this variable. Corroborating Ido *et al.* (2006) stated that rizotron is a viable framework for the study of the root system of sugarcane.

Table 8: Average levels of foliar nitrogen (plant cane and first ratoon) for the cultivars RB92579 and RB867515 under the different treatment conditions

Treatments	RB92579 (%)	RB867515 (%)
T1	0.87	0.87
T2	0.88	0.79
T3	0.86	0.79
T4	0.79	0.79
T5	0.88	0.80
T6	0.80	0.88

T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

Table 9: Population of bacteria *Burkholderia tropica*, *Gluconacetobacter dizotrophicus*, *Herbaspirillum* spp. and *Azospirillum amazonse* in root samples collected 5 months after planting in ratoon cane for the diffrent treatment conditions of the cultivar RB92579

Treatments	*Burkholderia tropica*	*Gluconacetobacter dizotrophicus*	*Herbaspirillum* spp.	*Azospirillum amazonense*
T1	25×10^{-5}	0.7×10^{-5}	2.5×10^{-3}	6.5×10^{-5}
T2	2.5×10^{-3}	6.5×10^{-5}	2.5×10^{-3}	4.5×10^{-5}
T3	4.0×10^{-7}	2.5×10^{-3}	2.5×10^{-3}	2.5×10^{-3}
T4	2.5×10^{-3}	2.5×10^{-3}	2.5×10^{-3}	0.9×10^{-3}
T5	2.0×10^{-7}	0.4×10^{-3}	2.5×10^{-3}	2.5×10^{-3}
T6	2.5×10^{-3}	6.5×10^{-5}	2.5×10^{-3}	4.5×10^{-5}

*Population based on the calculation of Most Probable No. (MPN) table according to McCrady (Dobereiner, 1992), T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

Table 10: Population of bacteria *Burkholderia tropica*, *Gluconacetobacter dizotrophicus*, *Herbaspirillum* spp. and *Azospirillum amazonse* in root samples collected 5 months after planting in ratoon cane for the diffrent treatment conditions of the cultivar RB867515

Treatments	*Burkholderia tropica*	*Gluconacetobacter dizotrophicus*	*Herbaspirillum* spp.	*Azospirillum amazonense*
T1	4.0×10^{-5}	0.7×10^{-5}	2.5×10^{-3}	2.5×10^{-3}
T2	25×10^{-5}	25×10^{-5}	2.5×10^{-3}	2.5×10^{-3}
T3	6.5×10^{-5}	2.5×10^{-3}	2.5×10^{-3}	6.5×10^{-5}
T4	4.5×10^{-5}	4.5×10^{-5}	6.5×10^{-5}	25×10^{-5}
T5	25×10^{-5}	1.5×10^{-5}	6.5×10^{-5}	7.5×10^{-7}
T6	4.5×10^{-5}	6.5×10^{-5}	2.5×10^{-3}	4.5×10^{-5}

Population based on the calculation of Most Probable No. (MPN) table according to McCrady (Dobereiner, 1992), T1: Non-inoculated, T2: Inoculated, T3: Non-inoculated and 80 kg N ha^{-1}, T4: Inoculated and 80 kg N ha^{-1}, T5: Non-inoculated and 14 Mg filter cake ha^{-1} and T6: Inoculated further 14 Mg filter cake ha^{-1}

CONCLUSION

The filter cake allowed an increased root growth, especially in the initial phase of the cycle which may have resulted from the retention of a greater number of stalk, thus resulting in a higher TCH (yield) and TPH. It was observed a significant correlation of length and volume of the root system with TCH and with the number of stalk. The method used to estimate the decal root length was effective when compared to the method of image analysis.

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