Plant Soil
DOI 10.1007/s11104-009-0059-1

REGULAR ARTICLE

Effect of diazotrophic bacterium inoculation and organic fertilization on yield of Champaka pineapple intercropped with irrigated sapota

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Received: 30 October 2008 / Accepted: 28 May 2009

Abstract The purpose of this study was to evaluate the response of the 'Champaka' pineapple to inoculation with the diazotrophic bacterium *Asaia bogorensis* (strain 219) when grown with organic fertilizer in an irrigated sapota orchard. Plantlets were transplanted to tubes containing a mixture of worm compost and vermiculite and inoculated with 10^8 bacterial cells. After five and a half months of acclimatization the plantlets were transplanted in furrows in the sapota orchard. Fertilizer was placed at the bottom of the furrows and covered with three doses (2.5; 5.0 and 7.5 L linear m^{-1} row) of three organic composts. The successful association of the plantlets with the diazotrophic bacterium was confirmed by most probable number analysis before transferring to the field. Plants inoculated with strain AB219 showed the greatest initial leaf growth and produced the heaviest fruits compared to uninoculated plants. Plant growth and fruit yield increased with increasing compost dosages. The results suggested that 'Champaka' pineapple benefited from the association of *A. bogorensis* (strain 219) when grown under irrigation and with organic fertilizer.

Keywords *Ananas comosus* · *Asaia bogorensis* · Organic fertilization · Plantlet inoculation · Cultural system

Responsible Editor: Euan K. James.

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Introduction

Pineapple is cultivated in tropical regions of developing countries, which produce 98% of all commercial pineapple. On the global scale, Brazil accounts for 13.4% of world production: roughly 2,5 million tons of fruit in 2006; second only to Thailand (FAO 2008). The northeastern region within the states of Brazil is responsible for 41% of the country's production (IBGE 2008).

Despite the high production in the Northeast of Brazil, where there are favorable climatic conditions for the culture, together with production infrastructure such as irrigation system, roads and port structure for disposal of production and government support for fruit export, there are still problems with growing the plant propagules (ground shoot, stem shoot and slips) since they are collected directly from fields of varieties susceptible to diseases. Varieties such as

Published online: 16 June 2009

'Perola' (Pernambuco group) and 'Smooth Cayenne' are produced, because of their ready acceptance in the internal market (Bengozi et al. 2007; Miguell et al. 2007) whereas the 'Cayenne Champac' (Champaka, Golden or MD-2), whose fruits have been exported from states such as Ceará, where production was minimal a few years ago (IBGE 2008). All these varieties are commonly sensitive to the fungus *Fusarium subglutinans* (Wollenw. & Reinking), the agent of brown rot, although the 'Pérola' and 'Smooth Cayenne' varieties are worse affected (Reinhardt et al. 2002; Santos et al. 2002).

Agro-chemicals have been used to overcome the susceptibility to disease, but they do not always effectively control brown rot (Santos et al. 2002). To counteract disease susceptibility the use of resistant genotypes (Borrás et al. 2001; Reinhardt et al. 2002) and healthy plantlets, which may be obtained by micro-propagation (Teixeira et al. 2001; Firoozabady and Gutterson 2003) together with quick propagation techniques in the nurseries are recommended.

An association of diazotrophic endophytic bacteria with pineapple plants has been reported (Weber et al. 1999; Tapia-Hernández et al. 2000). These beneficial microorganisms can be lost during the production of cuttings in vitro. Thus, inoculation of pineapple plantlets could represent an improvement of plantlet quality, since *Asaia bogorensis* and *Burkholderia*-like bacteria have been shown to promote plant growth during the acclimatizing phase (Weber et al. 2003a, b) and to increase fruit yield (Weber et al. 2004). In addition, some of these bacteria are antagonistic to *Fusarium* spp., according to preliminary tests (data not shown) and could therefore be an additional advantage to organic farming.

The preference for organic pineapples was observed amongst Dutch consumers (Poelman and Mojet 2003; Poelman et al. 2008) and is not likely to be any different for consumers from other nations. The production of organic pineapple is small, relatively undeveloped for commercial profit, and restricted to countries with little production (UNCTAD 2003). In Brazil, the organic system is confined to small areas, and the export of fresh or dried organic pineapples amounts to only 0.01% of all organic products (Brazil, Ministério do Desenvolvimento, Indústria e Comércio Exterior (2008b).

The organic system, used with arboreous species, including legumes, was adopted for the study of the 'Criolla' pineapple in the Nayarit region of Mexico (Rios-Torres and Uriza-Avila 2005). In an earlier study the application of organic material in a Regosol area (Gadelha et al. 1982) increased the size of the 'Pérola' pineapple fruit, but, in a recent study the cv 'Mauritius' pineapple did not respond to organic fertilization in soils of medium fertility (Devadas and Kuriakose, 2005).

A mixed system with interspersed planting (UNCTAD 2003; Haggar et al. 2003; Uriza-Ávila et al. 2005; Olanyan and Fagbayide 2007) led to an increase in soil productivity. The management of organic fertilizers could possibly be adopted in this multi-crop system, to satisfy both economic and environmental concerns.

The purpose of this study was to evaluate the response of the 'Champaka' pineapple to inoculation with the diazotrophic bacterium *A. bogorensis* (strain 219) when grown with organic fertilizer in an irrigated sapota orchard.

Material and methods

Plant material

Micro-propagated plantlets were formed from axillary shoots obtained from 'Champaka' pineapple plants (Weber at al. 2004). After sterilization in solution with 1% sodium hypochlorite, cuttings were cultured on MS medium (Murashige and Skoog 1962), to which agar (5 g L^{-1}) was added. They were then allowed to proliferate in the same medium, containing benzylaminopurine (1.0 mg L^{-1}) and naphthalene acetic acid (0.1 mg L^{-1}), and finally elongated in naphthalene acetic acid (0.2 mg L^{-1}) amended MS medium. Plantlets bearing 4 or 5 leaves (6 to 8 cm) were transplanted to 288 cm^3 tubes containing a mixture of worm compost and expanded vermiculite of medium texture (ratio of 2:3 v:v) and kept in a greenhouse with an automated intermittent spraying system. The chemical composition of the tubes substrate (Silva 1999) is shown in Table 1.

Microbial inoculation and plantlet acclimatization

Upon transplanting to the tubes, the plantlets received 1 ml liquid aliquots of Dygs medium containing 10^8 cells of *Asaia bogorensis* (strain AB219). The

Table 1 Mean values of the physical and chemical characteristics of the substrate used in the acclimatization of the plantlets and of the organic composts as well as the ash used in the field experiment

Characteristic	Unit	Substrate	Compost[1]				Ash
			A	B	C	Average	
Bulk density	kg l^{-1}	-	0.69±0.1	0.57±0.1	0.66±0.1	0.64±0.1	0.61
Moisture,	%	23.00	34.37±4.1	43.43±3.1	41.67±3.2	39.82±3.5	7.2
pH in water (1:2.5)	-	8.01	8.23±0.7	9.10±1.0	8.07±0.7	8.47±0.8	-
Electric conductivity	dS m^{-1}	2.90	4.23±3.6	1.87±0.6	3.27±1.5	3.12±1.9	-
Organic carbon	g kg^{-1}	80.30	292.67±66.5	383.33±88.3	321.00±82.7	332.33±79.2	-
N	g kg^{-1}	5.38	10.07±0.7	9.20±2.5	10.70±3.9	9.99±2.4	-
P	g kg^{-1}	14.30	9.63±0.2	4.13±1.3	6.33±1.4	6.70±1.0	2.5
K	g kg^{-1}	14.60	13.00±3.6	17.57±10.8	11.90±2.2	14.16±5.5	32.1
Ca	g kg^{-1}	8.33	37.87±18.1	21.83±8.8	26.47±4.1	28.72±10.3	70.2
Mg	g kg^{-1}	34.43	5.83±1.9	11.20±5.7	8.00±0.4	8.34±2.7	7.9
S	g kg^{-1}	7.54	10.20±0.2	10.63±0.4	8.83±3.2	9.89±1.2	-
Na	mg kg^{-1}	0.22	0.33±0.1	0.34±0.1	0.27±0.1	0.31±0.1	2300
Fe	mg kg^{-1}	36608.0	6953.3±2290	1565.0±167	12431.3±9641	6683.2±4033	636.3
Mn	mg kg^{-1}	30.00	624.03±364.3	433.10±32.4	481.43±92.7	512.86±16	206.4
Cu	mg kg^{-1}	9.40	23.07±11.8	21.93±8.2	21.77±5.2	22.26±8.4	40.1

(1) Organic fertilizers from three different seasons (May 2005, February and September 2006)

inoculum preparation was according to Weber et al. (2003b) who previously isolated and identified the bacterial strain from 'Smooth Cayenne' stems. Two control treatments were established, one group of plantlets was treated with Dygs medium and another group was given only sterilized distilled water. Tubes with treated plantlets were randomly distributed on tables in a greenhouse.

After four and one half months' cultivation, fresh and dry weights of roots and aerial parts of six representative plantlets of each treatment were recorded, and the fresh samples were analyzed for colonization by diazotrophic bacteria. Roots and stems were surface sterilized in 1% chloramine-T for 5 min, macerated with mortar and pestle, and the homogenate was serially diluted up to 10^{-7} and inoculated into vials containing semi-solid medium JNFb (Döbereiner et al. 1995) followed by growth at 30°C. Pure bacterial isolates from positive vessels were tested for their ability to grow on different carbon sources (sucrose, D-glucose, D-fructose or mannitol), using semi-solid JNFb medium without malate to identify the diazotrophic bacteria (Weber et al. 2003b). The most probable number (NMP) of diazotrophs was recorded for all treatments.

The performance of the treated pineapple plants

Following acclimatation inoculated plantlets were transplanted (September 2005) to an irrigated area, fertilized with different doses and sources of compost within a sapota orchard in the Irrigation District of Baixo-Acaraú, Ceará state, Brazil (Lat. 03° 03′ S and Long. 40° 04′ W, 36–55 m Alt.). The location is characterized by a hot and humid AW' climate with a rainy season from January to June, according to the Köppen classification, with average temperatures and relative humidity values of 28º and 70%, respectively, and sandy and deep Haplic Arenosols (Santos et al. 2005). The area was fallow since the removal of scrub vegetation in 2004, was managed organically and surrounded by a corridor of approximately 6 m with spontaneous vegetation forming a barrier to other cultures. The surface soil chemical characteristics (Van-Raij et al. 2001) were as follows: total acidity (H + Al)=11.6 $mmol_c$ dm^{-3}; organic material (oxidation-reduction)=17.7 gdm^{-3}; P (resin)=7.5 mg dm^{-3}; Na^+ (Merlich)=7.0 $mmol_c$ dm^{-3}; K^+, Ca^{+2} and Mg^{+2} (resin)=1.5; 18.0 and 6.0 $mmol_c$ dm^{-3}; Cu, Fe, Mn and Zn (DTPA)=0.1; 12.0; 13.6 and 1.8 mg dm^{-3} and 0.27 dS m^{-1} electric conductivity.

The area was furrowed to a depth of 0.4 m, leaving 1.4 m between rows. Areas were laid out (6×5.6 m), between sapota lines, and treated with three doses (2.5; 5.0 and 7.5 L linear m^{-1}) of three organic composts, consisting of: a) bovine manure, shredded leaves of wax palm (*Copernicia cerifera*) and sugar cane bagasse, b) plant debris and phosphate rock powder and c) sugar cane bagasse, coconut fiber, bovine manure, phosphate rock powder, rock powder (MB-4) and fruit residues of West Indian cherry (*Malpighia emarginata* DC). Each treatment was repeated threefold. Unfertilized control areas of the same size were left between two experimental units.

Following the fertilization (May 2005), furrows were covered with surface soil and irrigated by a Naan-Tif piping system of 16 mm diameter with every drip nozzle placed at 0.4 m intervals to reach two plants. Three double rows (0.3 and 1.1 m) of pineapple were established on the fertilized lines between the areas (5.6×6 m), plantlets inoculated with *A. bogorensis* (strain AB219) were distributed in two continuous rows with control plants in the neighboring rows, leaving 0.4 m between the plants within the rows and two plants per drip nozzle. Irrigation was carried out through the entire production cycle, providing the plants, in the dry and rainless periods, up to 7 mm water per day, which is the plant's water demand in the region (Santos et al. 2005).

During the plant growth phase, surface fertilizer was added twice (April and September 2006), applying the same doses and sources of composts to the base of the plants. The chemical composition of these composts is shown in Table 1.

Other common practices of organic production were adopted, such as manual weeding (once a month) between the rows of pineapple; ant repellent, neem leaves and fruits (*Azadirachta indica*) dispersed near the ant nest entrances; spraying with neem-based fermented extract (5 to 10 kg of green leaves and plant seeds in 100 L of water) to repel insect-born diseases, such as the pineapple mealy bug (*Dysmicoccus brevipes*) during the flowering period, and spraying in the fruit bearing period with an ash based suspension (4.2 kg suspension in 100 L of water, 3 mL per plant) (Table 1). In contrast to what is recommended for organic production, flower induction was carried out 18 months after planting (March 2007), using Ethrel (27.5±2.5 mL of commercial product to 1,500 mg L^{-1}), in order to obtain uniform flowering and fruit harvesting in the same period for all treatments.

The field evaluations consisted of leaf length at 5, 11 and 17 months after planting, estimation of the diazotrophic bacterial populations associated with the roots after 17 months, and yield. The evaluations were performed just before the fertilization in covering practice; harvesting was after 20 months, experiencing a peak at 24 months after the planting plantlets. The following fruit categories were established: 1) ≤900 g, 2) >900 g≤1,200 g, 3) >1,200 g≤1,500 g, 4) >1,500 g≤1,800 g, 5) >1,800 g≤2,100 g, 6) >2,100 g≤2,400 g, 7) >2,400 g, according to the yellow pulp pineapple classification (Brazil, Ministério da Agricultura, Pecuária e Abastecimento (2008a).

Statistical analysis

A completely randomized design was used to evaluate plantlet characteristics in the greenhouse experiment. The field experiment was laid out in a randomized block design as a split-plot, where sources and doses of compost were plots and types of plants were subplots.

The analyses of variance were performed for all evaluated variables, except frequency of fruit in each weight category, using the GLM procedure (General Linear Models) of the SAS® System (SAS Institute 2000). Comparison of average values of different organic compost sources and presence/absence of diazotrophic bacteria was achieved by using t-tests for contrasts. The effect of the compost doses was evaluated by linear regression.

Results

The performance of the pineapple treated plants in greenhouse

The micro-propagated 'Champaka' pineapple plantlets were successfully colonized by the potential diazotrophic bacterium as shown by the growth during the acclimatization phase in tubes containing worm compost and vermiculite (Table 2). Bacterial cultures isolated from roots and stems of inoculated plants grew in semi-solid N-free media containing malate, sucrose, D-glucose, D-fructose or mannitol as sole carbon source, as we observed with the strain AB219. The strain persistence on the plants should be considered in

Table 2 Population of diazotrophic bacterium *A. bogorensis* (strain 219) and dry biomass of the 'Champaka' pineapple, after four and a half months of cultivation in the greenhouse

Plant	NMP of diazotrophic bacteria (log g^{-1})				Dry biomass (g $plant^{-1}$)					
	Root	I[1]	Aerial part	I	Root	I	Aerial part	I	Total	I
Inoculated	3.165	15.1	2.401	30.2	1.305	17.4	11.340	23.1	12.645	22.5
Control 1	2.828	-	1.867	-	1.136	-	9.902	-	11.038	-
Control 2	2.672	-	1.822	-	1.087	-	8.518	-	9.605	-
Coefficient of variation (%)	6.27		12.98		15.89		8.99		8.44	

(1) Increment = [100 (X – Y) Y^{-1}], where X is the inoculated plant and Y is the average of the controls

future studies, since we observed a high colonization of roots (15.1%) and stems (30.2%) (Table 2). Differences from the controls are shown in Table 3.

Significant growth stimulation by *A. bogorensis* (strain AB219) was observed (Table 2) as determined by test t (Table 3). However, non-inoculated plants maintained a healthy appearance that allowed them to be transplanted to the field.

Performance of inoculated pineapple plants in the field

There was a successful crop establishment, so that most pineapple plants survived in the field (>99%, data not shown). In the first five months of transplanting, plantlets exhibited a striking yellowing, probably due to the exposure to direct solar radiation during the dry season (September to December 2005). After that period (February 2006), which coincided with the beginning of the rainy season, plants became dark green with variable leaf lengths (Table 4), according to the type of plantlet ($p<0.001$) and the doses of compost used (Table 5).

The most intense leaf growth was observed from the eleventh to the seventeenth month after transplantation on plantlets inoculated with *A. bogorensis* (strain AB219) and that received the higher compost dose (Tables 6 and 7).

After the 17th month in the field, pineapple plants showed very high and similar populations of potential diazotrophic bacteria in the roots (10^6 cells per gram of fresh weight), independent of their greenhouse treatments (Tables 4). Nevertheless, the root colonization by potential diazotrophic bacteria was positively influenced by compost C (Tables 4 and 6). The bacteria were isolated from inoculated plant roots and showed growth responses similar to those observed previously for the wild-type AB219 strain grown in semi-solid N-free medium, suggesting the presence of *A. bogorensis* in field-grown plants.

The type of compost had little influence on the leaf growth and it did not affect the yield (Tables 4 and 6). A small difference in leaf length observed after five months between sources A and C ($p=0.121$) (Table 6) was presumably due to the small variation in nutrient content among composts (Table 1).

Table 3 Estimates of the differences between means (D) and *p* values associated with the t test for contrasts, for the population of potential diazotrophic bacterium *A bogorensis* (strain 219) and the dry biomass of the 'Champaka' pineapple, after four and a half months of cultivation in the greenhouse

Plant	NMP of diazotrophic bacteria				Dry biomass					
	Root		Aerial part		Root		Aerial part		Total	
	D (log g^{-1})	*p*	D (log g^{-1})	*p*	D (g)	*p*	D (g)	*p*	D (g)	*p*
Inoculated vs. Control 1	0.336	0.063	0.535	0.047	0.169	0.311	1.438	0.095	1.607	0.080
Inoculated vs. Control 2	0.493	0.015	0.579	0.036	0.218	0.203	2.822	0.008	3.040	0.007
Inoculated vs. Controls	0.414	0.017	0.557	0.024	0.193	0.194	2.131	0.014	2.324	0.012

Table 4 Leaf lengths, population of potential diazotrophic bacteria in roots, fresh weight and the production of 'Champaka' pineapple, as a function of the different sources and compost dosages and the presence or absence of inoculation with *A. bogorensis* (strain AB219)

Treatments			Cultivation period (months)			NMP of diazotrophic bacteria in roots	Fresh weight of pineapple	Produtivity[2]	
Compost		Plantlet	Five	Eleven	Seventeen				
Source	Dosage		Lengthening of leaves (cm)			(log g^{-1})	(kg)	(t ha^{-1})	I[3]
A			28.23	43.93	61.37	6.016	1.174	31.452	-
B			28.24	44.76	64.97	6.187	1.155	30.944	-
C			30.16	46.44	65.20	6.429	1.191	31.911	-
	V1[1]		27.27	41.47	59.81	6.184	1.037	27.767	-
	V2		29.06	45.36	64.64	6.148	1.203	32.219	16.0
	V3		30.29	48.29	67.09	6.300	1.281	34.321	23.6
		Inoculated	30.50	47.96	64.78	6.328	1.219	32.661	6.0
		Control 1	28.88	44.59	63.36	6.172	1.144	30.637	-
		Control 2	27.25	42.58	63.40	6.133	1.158	31.009	-
Coefficient of variation (%)			(9.26)	(13.13)	(9.26)	(13.13)	(10.02)	(9.02)	(8.67)

(1) Volumes applied (m^3) up to five (13.39; 26.79 and 40.18), eleven (26.78; 53.57 and 80.35) and seventeen months (40.17; 80.35 and 120.50) after planting

(2) Production of 26,785 plants

(3) Increment=[100 (X–Y) Y^{-1}], where X is the plant that is inoculated or fertilized with high doses of compost and Y is the average of the controls or plants with low compost dose

Conversely, compost dosages influenced leaf growth and pineapple production (Tables 4, 5 and 7). Yield was largely increased (23.6%) when high compost doses were applied (120.5 m^3 ha^{-1}) (Table 4). Nonetheless, there was also a high frequency of small fruits (23%≤900 g and 55%≤1.200 g) (Fig. 1a).

The presence of *A. bogorensis* (strain AB219) affected the weight of fresh fruit (Table 6) as the

Table 5 Mean square (ms) and *p* values associated with the F test for leaf length, population of potential diazotrophic bacteria on roots and the fresh mass of the 'Champaka' pineapple fruits, as a function of fertilization with different sources and doses of compost and the utilization of plantlets inoculated and not inoculated with *A. bogorensis* (strain AB219)

Characteristic		Lengthening of leaves						NMP of diazotrophic bacteria on roots		Fresh weight of pineapple	
	GL	Five months		Eleven months		Seventeen months					
		ms (cm)	*p*	ms (cm)	*p*	ms (cm)	*p*	ms (log g^{-1})	*p*	ms (kg)	*p*
Source	2	33.384	0.201	44.049	0.776	124.555	0.516	1.161	0.051	0.009	0.898
Dosage	2	62.065	0.063	316.503	0.189	369.799	0.162	0.170	0.560	0.421	0.019
Source vs dosage	4	9.274	0.741	211.132	0.336	225.911	0.330	0.270	0.521	0.080	0.448
Plant	2	71.053	<0.001	199.054	0.007	17.694	0.652	0.287	0.409	0.044	0.023
Source vs plant	4	13.103	0.144	36.223	0.403	14.274	0.843	0.307	0.432	0.012	0.330
Dosage vs plant	4	3.961	0.697	25.197	0.584	30.414	0.569	0.179	0.687	0.009	0.472
Source vs dosage vs Plant	8	11.688	0.149	27.810	0.611	28.765	0.687	0.213	0.707	0.005	0.861

Table 6 Estimates of the differences between means (D) and *p* values associated with the t test for contrasts for the variables leaf length, population of potential diazotrophic bacteria in the roots and the fresh weight of the 'Champaka' pineapple fruits, as a function of fertilization with different sources and doses of compost and the utilization of plantlets inoculated and not inoculated with *A. bogorensis* (strain AB219)

Contrasts		Lengthening of leaves						NMP of diazotrophic bacteria in roots		Fresh weight of pineapple	
Compost	Plantlet	Five months		Eleven months		Seventeen months					
		D (cm)	*P*	D (cm)	*p*	D (cm)	*p*	D (log g^{-1})	*p*	D (kg)	*p*
A vs B		−0.015	0.990	−0.825	0.820	−3.600	0.340	−0.171	0.284	0.019	0.810
A vs C		−1.933	0.121	−2.506	0.491	−3.830	0.311	−0.413	0.017	−0.017	0.828
B vs C		−1.919	0.123	−1.681	0.643	−0.230	0.951	−0.242	0.137	−0.036	0.649
	Inoculated vs Control 1	1.619	0.032	3.365	0.044	1.419	0.420	0.156	0.313	0.076	0.010
	Inoculated vs Control 2	3.244	<0.001	5.374	0.002	1.385	0.431	0.195	0.209	0.062	0.032
	Inoculated vs Controls	2.431	<0.001	4.369	0.003	1.402	0.358	0.176	0.192	0.007	0.069

productivity was increased by 6% (Table 4). However, the bacterium provided little change in the frequency of fruit in weight categories (Fig. 1c), as 30% of the fruit did not reach 900 g, compared to fruits of control treatments 1 and 2 (35 and 32%). In addition, differences in the numbers of fruit were observed between plantlets (Fig. 1c), sources (Fig. 1a) and compost doses (Fig. 1b).

Discussion

The colonization of 'Champaka' plants by *A. bogorensis* (strain AB219) was observed in this study, confirming previous reports (Weber et al. 2003a, b, 2004). The presence of the potential diazotrophic bacterium was also detected in the uninoculated control plants. This may be due to endophytic colonization, apart from possible contamination of plantlets after transplantation into tubes.

The bacterial population associated with plant roots, grown in the greenhouse, was considered low (1.462 cells per gram of fresh mass) compared with the plantlet inoculation, which could be due to the application method of the inoculants on plant roots, soon after their transplantation into tubes. Weber et al. (2003b) observed an intense colonization of the roots (up to 10^6 bacteria per gram of fresh mass) after four

Table 7 Parameter estimates (intercept and slope) of the linear regression models used to describe the variation in the foliar length, population of potential diazotrophic bacteria on the roots and the fresh weight of the 'Champaka' pineapple fruits, as a function of the compost dosages applied in the different periods of the plantation

Variable	Cultivation period (months)								Fresh weight of pineapple	
	Five		Eleven		Seventeen					
	Lengthening of leaves						NMP potential diazotrophic bacteria in roots			
	D (cm)	$P^{(2)}$	D (cm)	*p*	D (cm)	*p*	D (log g^{-1})	*p*	D (kg)	*p*
Intercept	25.860	-	38.218	-	56.573	-	6.095	-	0.929	-
Slope	0.113	0.003	0.127	0.006	0.091	0.003	0.001	0.440	0.003	<0.001

(1) Volumes (m^3) applied up to five (13.39; 26.79 and 40.18), eleven (26.78; 53.57 and 80.35) and seventeen months (40.17; 80.35 and 120.50) of plantation

(2) P value associated to the t test utilized for the no dose effect hypothesis

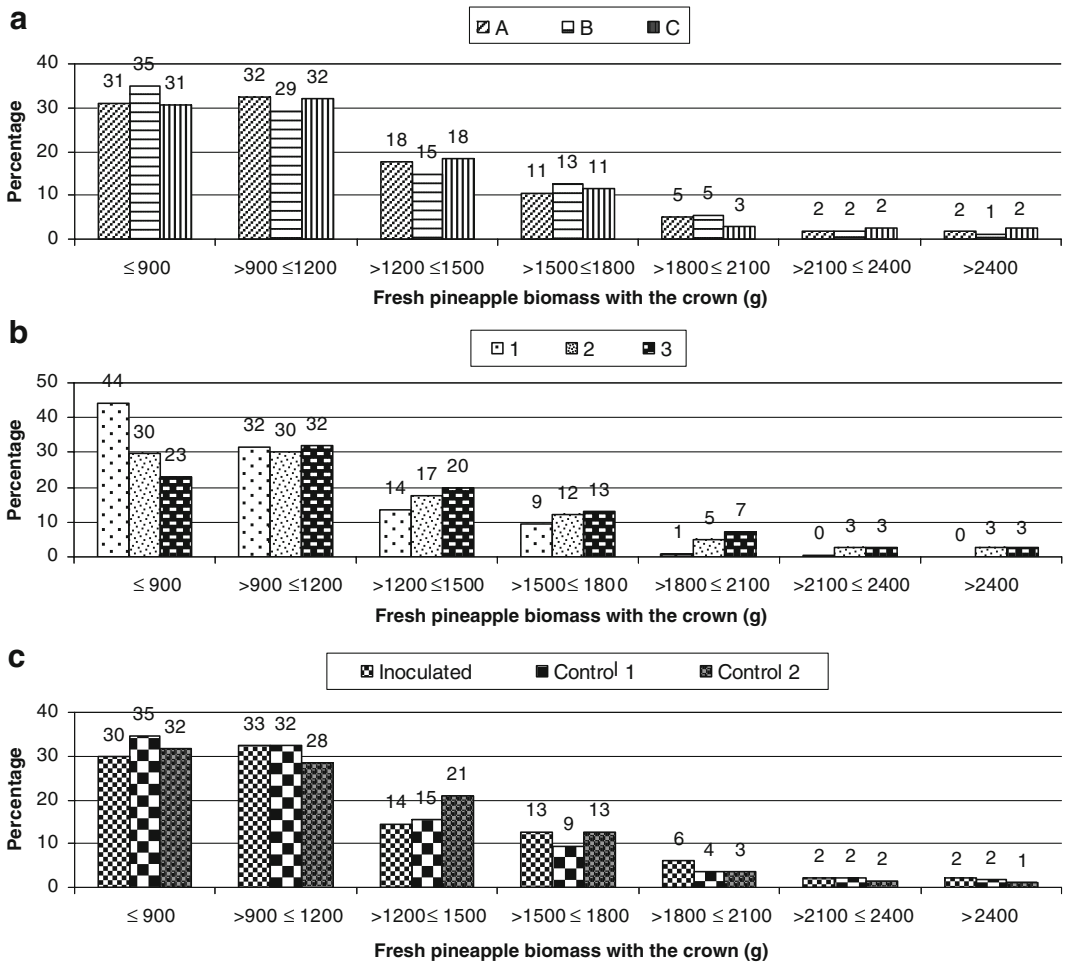

Fig. 1 Relative frequency of the 'Champaka' pineapple fruit in each one of the size classes, based on the fresh weight of the fruit, as a function of fertilization with different sources (**a**) and dosages of compost (**b**) and of the inoculation with *A. bogorensis* (strain AB219) (**c**)

months acclimatization of 'Champaka' pineapple micro-propagated plantlet inoculated under laboratory conditions.

The pineapple growth induction was confirmed by the presence of *A. bogorensis* (strain AB219) in the plants. Nevertheless, even the growth of non-inoculated plants was satisfactory under greenhouse conditions, allowing their transplantation into the field after five and half months. This period is compatible with data reported by Teixeira et al. (2001), but it is known that micro-propagated pineapple plantlets often need to be cultivated in pots for a longer period under canvas and in greenhouses, before being suitable for transplanting.

Under field conditions, leaf growth increase was induced by *A. bogorensis* (strain AB219) and organic fertilizer. It should be noted that the effect of the bacterial isolate was more pronounced during initial stages of plant growth in the field. This may be related to the improvement of soil properties over the cultivation period and the process of bacterial colonization, so that at seventeen months of planting no large differences in the number of potential diazotrophic bacteria associated with plant roots were observed.

The lower differences in bacterial density observed among organic sources in the field may be explained by the microbial communities (not shown), the degree of compost degradation (darker color) and the presence of more organic residues in compost C: sugar cane bagasse, coconut fiber, bovine manure, phosphate rock dust, rock powder

(MB-4) and Indian cherry pulp residues, compared with the other organic sources.

Leaf length and yield were influenced by the doses of compost used, although there was a high frequency of small fruit, which may be explained by partial assimilation of nutrients provided by the composts. Nutrients supplied by the highest compost dose used (463.6 kg of N; 310 kg of P and 657.2 kg of K per hectare) surpass the quantities of nutrients N, P and K that are extractable by the pineapple plant (Souza 1999).

Regardless of crop yield response, the soil organic matter should be managed to obtain a balanced nutrient uptake and export by pineapple plants. According to UNCTAD (2003), 1.0 kg N; 0.2 kg P; 2.5 kg K; 0.3 kg Ca and 0.1 kg Mg is exported per ton of produced pineapple fruits. Moreover, it must be recognized that organic fertilization improves the physical, chemical and biological properties of the soil (Bulluck et al. 2002, Tejada et al. 2006; Sampaio et al. 2008), which should encourage plant growth.

The efficiency of organic fertilization is influenced by the method of its application (2/3 of the doses were applied to the soil surface), and the irrigation system. The dip nozzle type of irrigation may not moisten the applied organic material sufficiently, thus reducing the rate of mineralization and nutrient uptake by plants. Gadelha et al. (1982), reported the production of cv 'Perola' fruits ranging from 1.2 to 1.4 kg weight by in-furrow-application of a full dose of organic material (600 g of chicken manure or 1,800 g of cattle manure or 1,800 g of sugar cane press sludge).

To our knowledge this is the first report demonstrating positive agronomic effects of *A. bogorensis* (strain AB219) on the productivity of 'Champaka' pineapple fertilized with compost in an interspersed mixed culture. Specific requirements of different plant species, such as spacing between species, should be considered whenever the system is applied to other agricultural crops (Olanyan and Fagbayide 2007).

Conclusion

The 'Champaka' pineapple benefited from the association with *A. bogorensis* (strain AB219) in the vegetative growth phase and in the production of fruit, under irrigation and organic fertilization.

Acknowledgements We thank Dr. Emilson Cardoso, Dr. Osmar Nickel and Dr. José Ivo Baldani from Embrapa and Dr. Geoff Yates for their valuable revision of the article. This study was partially supported by Bonafrux Organics and Bank of Northeast Brazil.

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