UTILIZATION OF BORON (^{10}B) DERIVED FROM FERTILIZER BY SUGAR CANE[1]

Henrique Coutinho Junqueira Franco[2], Paulo Cesar Ocheuze Trivelin[3], André César Vitti[4], Rafael Otto[5], Carlos Eduardo Faroni[6] & João Gabriel Tovajar[7]

ABSTRACT

The response to B in agricultural systems of sugar cane is still an unexplored issue; B application has however recently been widely publicized and used with a certain degree of frequency. The use of ^{10}B-labeled fertilizers may further contribute to clarify this practice. With the objective of evaluating sugar cane use of B (^{10}B) derived from fertilizer (boric acid), an experiment was conducted under field conditions in the 2005/2006 growing season. The experiment consisted of the installation of microplots (2 x 1.5 m) where 4 kg ha^{-1} B (boric acid with 85.95 % in ^{10}B atoms) dissolved in water was applied 90 days after planting (May 2005). The solution was applied to the soil on both sides of the plant row at a distance of 20 cm. After harvest (June 2006) the B content and ^{10}B abundance in % atoms in all parts of the sugar cane plants (stalks, dry leaves, tips and roots) were determined. Results showed that the total B accumulated was 471 g ha^{-1} in the entire plant (35 % in the stalks, 22 % in the dry leaves, 9 % in the tips and 34 % in the roots). The sugar cane plants used on average 14 % of the total accumulated B in the above-ground part

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[2] Agronomist, Post-doctoral candidate of Stable Isotopes Laboratory, Center of Nuclear Energy in Agriculture (Centro de Energia Nuclear na Agricultura), CENA/USP (FAPESP Scholarship holder). Av. Centenário 303, CP 96, Piracicaba-SP, Brasil. CEP 13400-970. E-mail: hjfranco@cena.usp.br

[3] Associate Professor, CENA/USP (CNPq Scholarship holder). E-mail: pcotrive@cena.usp.br

[4] Agronomist, Doctor in Sciences, Scientific Researcher of APTA-Pólo Centro Sul. Rod. SP 127, km 30, CP 28, Piracicaba-SP, Brasil. CEP13400-970. E-mail: acvitti@apta.sp.gov.br

[5] Agronomist, doctoral candidate of Graduate Program in Soils and Plant Nutrition of Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ/USP (FAPESP Scholarship holder). Av. Pádua Dias 11, CP 9, Piracicaba-SP, Brasil. CEP 13418-900. E-mail: rotto@esalq.usp.br

[6] Agronomist, Doctor in Soils and Plant Nutrition, researcher of Centro de Tecnologia Canavieira – CTC, Fazenda Santo Antônio, s/n, CP 162, Piracicaba-SP, Brasil, CEP 13400-970. E-mail: cfaroni@ctc.com.br

[7] Agronomist, COSAN SA unidade UNIVALE, Rod. Placido Rocha s/nº, CEP 16000-880, Valparaíso-SP, Brasil. E-mail: jgtoaliari@bol.com.br

(44 g ha^{-1}) and 11 % in the roots (19 g ha^{-1}), totaling 13 % in the entire plant (63 g ha^{-1}). The recovery of ^{10}B-fertilizer by sugar cane plants was low, around 2 % of the total applied amount.

Index terms: planted cane, stable isotopes, micronutrients, *Saccharum* spp.

RESUMO: *APROVEITAMENTO DO BORO (^{10}B) PROVENIENTE DO FERTILIZANTE PELA CANA-DE-AÇÚCAR*

No agrossistema da cana-de-açúcar a resposta à aplicação de B é ainda uma questão não esclarecida, porém é um procedimento que vem sendo utilizado com certa frequência. O uso de fertilizantes marcados com ^{10}B pode auxiliar no entendimento dessa prática. Com o objetivo de avaliar o aproveitamento do B (^{10}B) proveniente do fertilizante (ácido bórico) pela cana-de-açúcar, realizou-se um experimento em campo, na safra de 2005/2006, que consistiu na instalação de microparcelas (2 m de comprimento por 1,5 de largura), que, após 90 dias do plantio (maio de 2005), receberam 4 kg ha^{-1} de B (ácido bórico com 85,95 % em átomos de ^{10}B) dissolvidos em água. A solução foi aplicada ao solo nos dois lados da linha de plantio, a uma distância de 20 cm. Após a colheita (junho de 2006), foram determinados o teor de B e a abundância de ^{10}B em átomos % em todas as partes das plantas de cana-de-açúcar (colmos, folhas secas, ponteiros e raízes). Os resultados mostraram que o acúmulo total de B foi de 471 g ha^{-1}, e, dessa quantidade, 35 % estavam nos colmos, 22 % nas folhas secas, 9 % nos ponteiros e 34 % nas raízes. O aproveitamento do ^{10}B-fertilizante pela cana-de-açúcar representou em média 14 % do boro total acumulado na parte aérea (44 g ha^{-1}), 11 % nas raízes (19 g ha^{-1}) e 13 % na planta inteira (63 g ha^{-1}). A recuperação do ^{10}B-fertilizante pela cana-planta foi baixa, em torno de 2 % do total aplicado.

Termos de indexação: cana-planta, isótopos estáveis, micronutrientes, Saccharum *spp.*

INTRODUCTION

Among the crops used for the industrial production of ethanol, sugar cane, especially Brazilian sugar cane, stands out on the world scene as superior over, for example, corn alcohol, due to its high productivity and photosynthetic efficiency in the tropical environment (Rodrigues, 2004). Currently, with the clear perspective of an increasing global demand for renewable energy sources replacing petroleum, the Brazilian agricultural production of sugar cane should accompany this trend of worldwide growing ethanol demand.

The Brazilian sugar cane production in the 2007/2008 harvest was 528 million tons, on a crop area of 6.6 million hectares (CONAB, 2007), which represents an average productivity of 80 Mg ha^{-1}. This productivity is far from the potential of production of the varieties used in Brazil, which calls for research to provide a solution for the problems causing this production deficit.

In the sugar cane sector, historically little attention has been paid to the response of sugar cane to the application of micronutrients. Studies are scarce and results sometimes inconclusive. Recently, fertilizer companies have been promoting and recommending the application of micronutrients to crops (by application to the soil, in planting furrows or to leaves) in spite of the lack of scientific support proving the efficacy of these practices. Therefore, research is necessary to establish biological and chemical parameters underlying the micronutrient applications, in order to avoid excessive application, which may unnecessarily increase production costs.

Of the micronutrients, B is the most deficient in Brazilian soils and a growing number of crops present visual symptoms of B deficiency (Moreira et al., 2000). It is an essential element for plant growth, a constituent of diverse enzyme compounds and activators and participates in diverse processes such as ion absorption, carbohydrate transport, lignin, cellulose, nucleic acid, and protein synthesis (Alleoni et al., 1998). According to Römheld (2001), the main function of B is the stabilization of the cell walls and, presumably, of biomembranes by the complexometry of organic compounds of the cis-diol functionality. Consequently, B deficiency will result in the inhibition of apical root growth. Involvement of B in the metabolism of phenols and IAA may cause death in the points of growth and necrosis in new leaves. Excess B concentrations may lead to the participation of B in the metabolism of phenols and in IAA, resulting in death at the growth points and necrosis in new leaves.

The use of isotope tracers in research evaluating uptake, transport and redistribution within the plant, as well as plant use of mineral nutrients has been recognized as a useful and efficient technique in the understanding of these phenomena. According to Boaretto (2006), with the development of the ICP-MS (plasma source mass spectrometer) it has become possible to distinguish stable B isotopes (^{10}B and ^{11}B), which allowed the performance of these studies, principally of B mobility in plants (Brown & Hu, 1996, 1997, 1998), using ^{10}B-enriched compounds. Nevertheless, in worldwide literature there is no record of studies in which the ^{10}B isotope technique was used in sugar cane. Therefore, by the isotope dilution method, it is possible to measure ^{10}B-fertilizer utilization by sugar cane, evaluating the real efficiency of fertilization of the micronutrient, as well as the fate of B within the plant after root uptake. In addition, with the imposed condition of sugar cane harvesting without residue burning in the São Paulo State, Brazil, the study of the ^{10}B balance established in the plant compartments (dry leaves, stalks, tips and roots), will provide detailed information about exportation, cycling and fate of B-fertilizer in the soil-plant system.

The objective of this study was to evaluate B (^{10}B) utilization derived from fertilizer (boric acid) by sugar cane (planted cane) harvested without burning of the crop residues.

MATERIAL AND METHODS

The experiment was carried out in a commercial plantation of sugar cane (Latitude 21 ° 55 ' 54 " S, Longitude 47 ° 10 ' 54 " W, 650 m asl), of the São Luiz Sugar Mill (Usina São Luiz) located in Pirassununga County, State of São Paulo, Brazil. The climate is Aw (Tropical Savanna, by the Köppen classification). The area has a slight slope (< 10 %) and the soil was classified as medium texture Latossolo Vermelho-Amarelo distrófico (Embrapa, 2006), Typic Haplustox (Soil Survey Staff, 2003). The soil was chemically analyzed prior to the experiment (Table 1). It is noteworthy that the experimental area was chosen because the B soil content (Table 1) was very low (< 0.20 mg dm^{-3}) for soils in the São Paulo State (Raij et al., 1996).

The soil was prepared with two heavy diskings, before and after subsoiling, the first to eliminate the old ratoons and the second to incorporate 2 Mg ha^{-1} of dolomitic lime and 2 Mg ha^{-1} of agricultural gypsum (rates defined by the agricultural department of the Sugar Mill). The final soil preparation consisted of medium disking, before creating furrows. Sugar cane was planted between February 21 and 24, 2005.

At planting, urea, potassium chloride and simple superphosphate were applied at the bottom of the furrow, at rates of 80, 120 and 120 kg ha^{-1} of N, K_2O and P_2O_5, respectively. These rates were chosen based on the recommendation of the Boletim Técnico 100 (Technical Bulletin 100) (Espironello et al., 1996). The plant cuttings of sugar cane variety SP81 3250 (2 seedpieces per meter, containing 17 to 20 buds per meter) were placed at the bottom of the furrow (row spacing of 1.5 m) cut in seedpieces with 2 to 3 buds and then covered with soil. Ninety days after planting (May 24, 2005) four microplots were installed (2 x 1.5 m), covering a total of 3 m^2, where the boric acid labeled with ^{10}B (85.95 % in ^{10}B atoms) was applied. The rows adjacent to the row fertilized with ^{10}B received the application of the same B rate, however without labeled isotope (19.87 % in ^{10}B atoms). The B rate of 4 kg ha^{-1} was dissolved in water (mixture of 200 L ha^{-1}) and applied to the soil, on both sides of the planting row, at a distance of 0.20 m from the sugar cane plants.

Table 1. Chemical properties of soil samples from the experimental area obtained from sampling between the rows of the previous ratoon

Properties	Depth (cm)			
	0–25	25–50	50–75	75–100
pH ($CaCl_2$)	5.5	4.7	4.6	4.6
SOM (g dm^{-3})	20	13	10	8
P (mg dm^{-3})	9	6	2	4
K (mmol$_c$ dm^{-3})	2.4	1.0	0.8	0.7
Ca^{2+} (mmol$_c$ dm^{-3})	29	10	7	6
Mg^{2+} (mmol$_c$ dm^{-3})	8	3	3	3
H + Al (mmol$_c$ dm^{-3})	20	31	28	25
Al^{3+} (mmol$_c$ dm^{-3})	1	3	3	3
SB (mmol$_c$ dm^{-3})	39.4	14.0	10.9	9.7
CEC (mmol$_c$ dm^{-3})	59.6	44.8	38.6	34.7
V (%)	66	31	28	28
m (%)	3	18	22	24
S-SO_4 (mg dm^{-3})	6	45	68	74
Cu (mg dm^{-3})	1.2	0.9	0.6	0.4
Fe (mg dm^{-3})	25	20	11	7
Zn (mg dm^{-3})	0.4	0.4	0.3	0.5
Mn (mg dm^{-3})	3.4	0.4	0.3	0.5
B (mg dm^{-3})	0.17	0.18	0.14	0.12

pH : $CaCl_2$ 0.01 mol L^{-1} (Raij et al., 2001); SOM: soil organic matter, colorimetric method (Raij et al., 2001); P: extraction by ion exchange resin and determination by colorimetry (Raij et al., 2001); S: 0.5 mol L^{-1} NH_4OAc in 0.25 mol L^{-1} HOAc (Vitti, 1989); K: extraction by ion exchange resin and determination by flame photometry (Raij et al., 2001); Ca and Mg: extraction by ion exchange resin and determination by atomic absorption spectrometry (Raij et al., 2001); H + Al: determination by potentiometer in SMP-buffer solution (Raij et al., 2001); Al: 1 mol L^{-1} KCl; B: $BaCl_2.2H_2O$ 0.125 % microwave (Raij et al., 2001); Cu, Fe, Mn, Zn: DTPA-TEA pH 7.3 (Raij et al., 2001).

The experiment was harvested from June 7 to 10, 2006. The above-ground part of the plants of the microplots with B (^{10}B) was harvested manually from 1.0 m of the center row (CR) and in contiguous positions in adjacent rows (AR) to the microplot. Samples of dry leaves, tips and stalks were seperated, following the method described by Trivelin et al. (1994) for the stable isotope ^{15}N. In all samples, the mass of the natural plant matter was determined and then this material was chopped in a mechanical forage chopper. After grinding and homogenization of each fresh sample, a subsample was removed, which was dried in a forced air laboratory dryer at 65 ºC to determine humidity. Roots were sampled by a probe (55 mm inner diameter) in the center of the microplots, at a depth of 0–40 cm, following the method described by Otto et al. (2009), where two samples were removed from the planting row itself, two samples at a distance of 30 cm from the planting row and two samples 60 cm away from the planting row. In these samples, soil was separated from the roots by dry sieving in a 2.0 mm screen sieve. The roots and rhizomes separated from the soil were washed in running water, dried in a ventilated laboratory oven at 65 ºC and the dry matter measured. All dried samples were ground in a Wiley mill and the B concentrations (Malavolta et al., 1997) and the abundances of atoms % of ^{10}B (Bellato et al., 2001) were determined.

Recovery of fertilizer-^{10}B in the plant was calculated by means of the equations:

$$BPPF = \left(\frac{A - C}{D - C}\right) \text{ x } BT \qquad RB\ (\%) = \left(\frac{BPPF}{BAF}\right) \text{ x } 100$$

with BPPF – B in the plant derived from fertilizer-^{10}B; A – abundance of B (atoms %) in the plant; C – natural abundance of ^{10}B (19.87 % of atoms); D – abundance of ^{10}B (85.95 % of atoms) of fertilizer-B; RB – recovery percentage of fertilizer-^{10}B in the plant; BT – B content in the plant (g ha^{-1}); BAF – B rate of the applied source (4000 g ha^{-1} B).

Rainwater was collected in rain gauges installed beside the experimental area (Table 2).

RESULTS AND DISCUSSION

At planted cane harvest, 13 months after fertilizer-^{10}B application, there was an accumulation of around 160, 105, 40 and 160 g ha^{-1} B in the stalks, dry leaves, tips and roots, respectively (Table 3). The results indicated that the micronutrient was uptake in the initial growth stages of sugar cane, since 22 % of the total B of the plant was contained in the dry leaves (Table 3). The total B accumulation in the planted cane was 471 g ha^{-1}, with 308 and 163 g ha^{-1} B found in the above-ground part and roots respectively, emphasizing that B in the roots represented 34 % of the total accumulated B of the entire plant. This significant B accumulation in the roots is possibly related to the functions of this nutrient in the plant: stabilization of the cell walls and, presumably also of biomembranes by the complexing of the organic compounds of the cis-diol functionality. Consequently, B deficiency will result in the inhibition of apical root growth (Römheld, 2001).

On the other hand, many studies have recently been developed with the purpose of investigating B mobility in plants, remembering that among the essential elements for higher plants, B is the only one with significantly varying within-plant remobilization among species (Brown & Hu, 1996). For the redistribution of this nutrient, the plant must have compounds such as polyols (alcohols derived from sugars as sorbitol, mannitol and dulcitol) in the phloem. With the objective of finding sequences in the SugarCane EST Genome Project (SUCEST) data bank that codify enzymes active in the metabolic pathways of sorbitol and mannitol, Marino et al. (2003) verified, by means of the comparison of enzymes of other organisms, the similarity of 18 "contigs" of sugar cane (*Saccharum* spp.) with 11 enzyme sequences that compose the probable metabolic pathway of fructose-derived sorbitol and mannitol. Of these "contigs", the sequences of seven were highly similar. In this context, the high B quantities in the roots (34 % of total B of the planted cane) may be a result of the remobilization of the micronutrient from the above- to the below ground part of the crop. However, when B deficiency in sugar cane plants is severe, the symptoms of B lack occur in new leaves (Salgado et al., 2003), indicating that the nutrient is not very mobile in sugar cane.

The planted cane yield was 108 t ha^{-1} of stalks available for processing (TSP) (TSP: dry mass of stalks multiplied by moisture content of 73 %). Thus, the demand of the planted cane in B for the production of 100 TSP was 150, 285 and 436 g ha^{-1} B, respectively, considering the B accumulation in the stalks, in the

Table 2. Monthly rainfall measured in the experimental area during the crop growth cycle

Monthly rainfall (mm)											Monthly rainfall (mm)						Total
2005											2006						
Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	
20	192	33	57	11	18	1.0	56	79	56	233	177	320	202	53	3.0	0.0	1511

Table 3. Accumulation of total B and fertilizer-^{10}B use by the planted cane (stalks, tips, dry leaves and roots)

Parts of the plant	Row	DM	B content	Total B	^{10}B	BPPF	BPPF	RB	RB	
		kg ha^{-1}	mg kg^{-1}	g ha^{-1}	atoms %	%	g ha^{-1}	%	%	g ha^{-1}
Stalks	AR	27055	6.1 ± 0.8*	164 ± 30	20.39 ± 0.1	0.78 ± 0.2	2.6 ± 0.7	0.06 ± 0.01	0.46 ± 0.04	18.5 ± 1.5
	CR	31292	5.1 ± 0.3	161 ± 7	26.42 ± 0.5	9.91 ± 0.7	15.9 ± 1.6	0.40 ± 0.04		
Dry leaves	AR	8995	10.6 ± 0.4	96 ± 2	20.93 ± 0.2	1.59 ± 0.3	3.0 ± 0.5	0.08 ± 0.01	0.41 ± 0.5	16.3 ± 2.0
	CR	8099	13.8 ± 1.1	112 ± 2	27.66 ± 0.6	11.78 ± 1.0	13.2 ± 2.0	0.33 ± 0.05		
Tips	AR	3887	10.2 ± 0.6	40 ± 9	20.75 ± 0.1	1.33 ± 0.2	1.0 ± 0.3	0.03 ± 0.00	0.22 ± 0.01	8.8 ± 0.5
	CR	4438	9.9 ± 0.7	44 ± 3	31.53 ± 1.1	17.65 ± 1.7	7.8 ± 0.3	0.19 ± 0.01		
Roots	–	6073	26.8 ± 3.6	163 ± 22	27.31 ± 2.5	11.26 ± 1.8	–	–	0.48 ± 0.1	19.0 ± 5
Above ground	–	41883	–	308 ± 42	–	14.14 ± 1.7	–	–	1.09 ± 0.1	43.5 ± 3
Entire plant	–	47956	–	471 ± 51	–	12.99 ± 1.5	–	–	1.56 ± 0.2	62.5 ± 7

DM: Dry matter; AR: Adjacent row to the microplot; CR: Center row of the microplot; BPPF: boron in the plant derived from the fertilizer; RB: Recovery of fertilizer ^{10}B. *Standard deviation of the mean for n = 4.

above-ground part (stalks, dry leaves and tips) and in the entire plant (above-ground part and roots). This demand is well above that reported by Franco et al. (2008a) with the same variety (SP81 3250), planted in two areas in the São Paulo State, which needed only 140 g ha^{-1} B to produce 100 TSP, considering the uptake of the above-ground part. Soriano (2007) verified that 100 TSP exported by the stalks, in the mean of eight varieties (RB72 454, RB86 7515, RB97 1755, RB95 1541, RB93 1003, RB92 579, RB86 3129, and RB93 509), 122 g de B. Vitti et al. (2006) presented on extraction of 235 g B per 100 TSP, which is close to the result (210 g B per 100 TSP) of Malavolta (1994). Orlando Filho et al. (1980) claimed that for the production of 100 TSP, on average, 195 g B were removed by the stalks and 311 g B by the above-ground part.

At harvest, approximately 30 % of the total accumulated B by the entire plant was exported by the stalks of the planted cane (Table 3), which shows that most B uptake by the crop remained in the soil-sugar cane system, due to mechanical harvesting without residue burning (conservationist management "green sugar cane"). In this sense, around 150 g ha^{-1} remained in the residual trash (dry leaves and tips), an accumulation that may be reused by the crop in subsequent cycles, after the mineralization of this organic residue. Considering only B uptake in the above-ground part, exportation of B by the stalks represented 52 % of the total accumulated B. Similarly, Franco et al. (2008a) observed that the stalks of SP81 3250 exported, upon harvest of the planted cane, 54 % of the accumulated B in the above-ground part. Orlando Filho et al. (1980) reported only slightly higher values of B exportation by the stalks (CB41 76), in the range of 60 %.

The enrichment in atoms % of ^{10}B in plants of the center row (CR) was greater than in plants of the adjacent rows (AR); consequently, the BPPF (% and g ha^{-1}) of these samples was greater. The practical result of this fact is that 86 % of the total fertilizer-^{10}B in the above-ground part of the plants of the microplot was derived from fertilizer applied to the CR and the rest from the AR, indicating that sugar cane plants took up fertilizer applied to their own row, as well as fertilizer applied to the adjacent rows, due to the extension of the root system and the diffusion of fertilizer-^{10}B in the soil. These results corroborate those of Trivelin et al. (1994, 1995, 1996) for N, in studies with the use of the stable isotope ^{15}N in sugar cane.

The B distribution of the fertilizer in the planted cane was 30 % in the stalks, 26 % in the dry leaves, 14 % in the tips and 30 % in the roots (Table 3). This distribution is note very discrepant to that of total B (34 % in stalks, 22 % in dry leaves, 9 % in tips and 35 % in roots), suggesting that the distribution of fertilizer-^{10}B within the plant is similar to that of the total accumulated B.

The utilization of the fertilizer-^{10}B by sugar cane represented on average 14 % of the total accumulated B in the above-ground part (44 g ha^{-1}), 11 % of the total accumulated B in the roots (19 g ha^{-1}) and 13 % of the total accumulated B in the entire plant (63 g ha^{-1}). Based on these results, it may be stated that B in the plant derived from the fertilizer-^{10}B, quantified at sugar cane harvest, represented a small fraction of the total B accumulated by sugar cane. Therefore, the main B sources for sugar cane in the planted cane cycle must have been the B available in the exchangeable soil fraction and the mineralization of the native organic matter of the soil and of crop

residues recently incorporated in the soil at cane planting. Even with the low initial soil B contents (Table 1), sugar cane, which has a root system with a high nutrient uptake capacity and good distribution in the soil profile (Smith et al., 2005), is less demanding in micronutrients than other crops (Evans et al., 1955 cited by Cambria et al., 1989). And, according to Malavolta (2006), the principal B source for plants is organic material, from which the nutrient is released by mineralization together with N and S, passing to the soil solution. From there it is uptake by plants, adsorbed by clay minerals and Fe and Al oxides or lost through leaching and erosion.

Recovery of the fertilizer-^{10}B by the planted cane was low, around 2 % of the total applied amount, indicating the low agronomic efficiency of the applied B rate (4 kg ha^{-1}). Nevertheless, due to the lack of studies with ^{10}B in sugar cane in the world literature, the interpretation of the results of efficiency is difficult. Therefore, in the absence of studies of fertilizer-^{10}B use in sugar cane, those of Boaretto (2006) with irrigated orange crop (variety Valencia grafted on a Swingle citromelo rootstock) were used, to compare the results of recovery and agronomic efficiency. The author verified that an application of 1 kg ha^{-1} B (boric acid) also resulted in a low recovery of fertilizer-^{10}B in orange trees: 7 % (65 g ha^{-1} of ^{10}B) from soil application and 2 % (17 g ha^{-1} of ^{10}B) from leaf application. In spite of being another crop, these results show that even at low B rates, fertilizing efficiency is low, due to the low accumulated quantity of the element by the plants. Nevertheless, it should be highlighted that leaching losses of fertilizer-^{10}B may have contributed to the recovery results. In an experiment in soil columns, Rosolem & Bíscaro (2007) ascertained that B leaching is closely related to the soil nutrient contents and to the rate of applied fertilizer-B (5 kg ha^{-1} B), which is up to 10 times greater than the quantities found in the control.

The participation of fertilizer-^{10}B in the total B accumulated by sugar cane plants was approximately 15 %, considered a satisfactory value, in view of the fact that the stable isotope of N (^{15}N) in sugar cane is in the range of 10 to 20 % (Trivelin et al., 1994, 1995, 1996, 2002; Gava et al., 2001; Vitti et al., 2007a,b; Franco et al., 2008b). The BPPF determined by Boaretto (2006) varied with the form of application of the fertilizer-^{10}B to orange trees; values after soil application were in the range of 30 %, and after leaf application around 10 %.

In view of the lack of data of fertilizer-^{10}B recovery by sugar cane on a global level, and the unprecedented aspect of this study of using the ^{10}B isotope dilution technique for the crop, it is evident that the results presented here may not be interpreted absolutely unequivocally, above all if based exclusively on the efficiency of B application (2 % recovery). New studies are necessary to refine the B recommendation for sugar cane, mainly concerning the rate to be applied, but also to investigate the fate of the micronutrient in the soil-plant system, in fertilizer-^{10}B balance studies.

CONCLUSIONS

1. Fertilizer-^{10}B represented a small fraction (13 %) of the total B accumulated by sugar cane plants at planted cane harvest.

2. The fertilizer-^{10}B recovery (utilization) was around 2 %, indicating low agronomic efficiency of the applied B rate (4 kg ha^{-1}).

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