Note

NITROGEN-15 LABELING OF *Crotalaria juncea* GREEN MANURE

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ABSTRACT: Most studies dealing with the utilization of ^{15}N labeled plant material do not present details about the labeling technique. This is especially relevant for legume species since biological nitrogen fixation difficults plant enrichment. A technique was developed for labeling leguminous plant tissue with ^{15}N to obtain labeled material for nitrogen dynamics studies. Sun hemp (*Crotalaria juncea* L.) was grown on a Paleudalf, under field conditions. An amount of 58.32 g of urea with 70.57 ± 0.04 atom % ^{15}N was sprayed three times on plants grown on eight 6-m^2-plots. The labelled material presented 2.412 atom % ^{15}N in a total dry matter equivalent to 9 Mg ha^{-1} This degree of enrichment enables the use of the green manure in pot or field experiments requiring ^{15}N-labeled material.
Key words: ^{15}N -fertilizer, labeled legumes, green manure

MARCAÇÃO DO ADUBO VERDE *Crotalaria juncea* COM ^{15}N

RESUMO: A grande maioria dos estudos com a utilização de material vegetal marcado com o isótopo ^{15}N não apresentam detalhes tão importantes sobre como foram obtidos esses materiais. Em se tratando de marcação de leguminosas as dificuldades em se obter material marcado com ^{15}N são ainda maiores pelo fato de serem plantas fixadoras de nitrogênio. Isso posto foi estabelecida uma técnica de marcação de leguminosas com nitrogênio (^{15}N), com o objetivo de obter material vegetal marcado isotopicamente para estudos de dinâmica do nitrogênio. Cultivou-se a leguminosa crotalária júncea (*Crotalaria juncea* L.), em Argissolo Vermelho Amarelo distrófico, em campo. Ao se aplicarem via foliar 58,32 gramas de uréia em oito canteiros experimentais, (uréia com 70,57 ± 0,04% de átomos de ^{15}N) parceladas em três vezes, obteve-se material vegetal marcado seco que continha 2,412 % em átomos de ^{15}N em uma massa seca equivalente a 9 Mg ha^{-1}. Essa marcação permite o uso dessa massa vegetal em estudos de dinâmica de nitrogênio.
Palavras-chave: fertilizante-^{15}N, leguminosa marcada, adubo verde

INTRODUCTION

The use of the stable isotope ^{15}N can help to identify nitrogen sources and is important for research on nitrogen dynamics in the soil-plant system. The ^{15}N labeling of green manures allows the determination of the amount of the nutrient in the soil and in the subsequent crop derived from the green manure which is only feasible with the use of isotopic methods. High degree of labeling of legumes with ^{15}N is complicated since these plants usually obtain a significant part of their N from the air, through biological N fixation from either soil or inoculated bacteria. Most papers on ^{15}N do not explain how the leguminous plant material was marked. Ambrosano et al. (1997) established a ^{15}N labeling technique for legumes growing in a greenhouse, and obtained a dried material with 3.177 and 4.337 atom % ^{15}N, for velvet bean and sun hemp, respectively.

Ambrosano (1995), using the techniques later described by Ambrosano et al. (1997) for velvet bean and sun hemp, determined that 60 to 80% of plant nitrogen remained in the soil, 20 to 30% were absorbed by corn plants, and 5 to 15% were lost from the soil-plant system. Azam at al. (1985) investigated the incorporation of *Sesbania aculeata* residues labeled with atom % 0.617 in ^{15}N excess, and determined that only 5% of the N from the legumes was absorbed by the corn plants. In the balance calculated by the authors, losses were around 5% when only *Sesbania* was applied. However, the low labeling levels used by Azam et al. (1985) may have

affected the results. The accuracy of isotope assays is directly proportional to the labeling levels, i.e., the lower the labeling level, the lower the accuracy. For more accurate (Bartholomew, 1965) and trustable results labeling level of at least 2 atoms % ^{15}N excess is needed.

Little attention has been given to the effectiveness of green manures in supplying nutrients to crops (Muraoka,1984). The ^{15}N labeling technique provides more precise information on nitrogen dynamics in the soil-plant system. Once the green manure is labelled, the fate of the nitrogen release from the legumes can be traced.

Crotalaria juncea is widely distributed over the tropics. It grows as a shrub, with a straight trunk, and its fibers have high quality cellulose, adequate for paper and other uses. *Crotalaria* grows fast, and can reach 3.0 to 3.5 m height, with an average yield ranging from 10 to 15 Mg ha^{-1} of dry material when sown in the summer. Since it is considered a bad host for galls and cysts-forming nematodes, it is highly recommended as a green manure. The crop cycle can last 180 days, but, when grown as green manure, cutting is suggested at about 120 days, during the peak of flowering (Salgado et al. 1987).

Legumes are important in crop rotations including sugar cane. *Crotalaria* is usually chosen because of its high biomass production, high biological nitrogen fixation, and capacity for controlling nematode infestation (Mascarenhas et al., 1994). However, field experiments require large amounts of ^{15}N-labeled material and there is a lack of information on ways of producing ^{15}N-labaled legumes under field conditions. Therefore, the objective of the present study was to establish procedures for ^{15}N labeling techniques of *Crotalaria juncea* grown in the field.

MATERIAL AND METHODS

The IAC 1-2 variety of *Crotalaria juncea* L was used in this study and was grown with no fertilization in a Paleudalf in Piracicaba, SP, Brazil. *Crotalaria* was sown (25 seeds per meter) on December 4, 2000 and emerged nine days after. Seeding was delayed to mid summer to avoid high growth rates, which could lead to higher ^{15}N isotopic dilution.

The experimental site consisted of 12 plots containing 6 rows of *Crotalaria*, 2 m long, spaced 0.5 m. The 6 m^2 *Crotalaria* plots were placed in the middle of 140 m^2 plots which were planed to be grown subsequently with sugar cane. In an adjacent area *Crotalaria* was cultivated in the same manner so that dry matter yields could be periodically assessed without affecting the experiment.

Eight plots were used for the labeling the *Crotalaria* plants and four were left as control (T1). The eight labeled plots were divided in two groups or four plots, which were meant for different treatments in the sugar cane trial that would follow. Although they received the same amounts of ^{15}N-urea, these plots were referred to as T2 and T3. Initially 5 applications of ^{15}N-labeled urea were planned but only 3 applications were made because of the fast growth rate of *Crotalaria* and the very fast isotopic dilution could negatively affect the ^{15}N enrichment.

Urea (58.32 g), with 70.57% + 0.04 ^{15}N atoms % was used to label the 8 *Crotalaria* plots. For the first application 11.66 g of urea were diluted in 1000 mL of water and exactly 125 mL of this solution were sprayed to each plot. For the second and third applications, 2000 mL of solution, with the same urea concentration, were prepared, using 250 mL per plot. The ^{15}N-urea solutions were sprayed 29, 59 and 74 days after plant emergency. The first application was made with a small spray bottle (Dompel brand, 350-mL capacity) because the 29-day old plants (0.45 m high) presented a relatively small leaf area. For the other two applications a garden sprayer (Bruden brand, 4-L capacity) was used. During the foliar spray of urea, the soil and borders of the plots were covered with plastic sheets to avoid contamination of the soil and the surrounding plants. The dates of urea application as well as the height and plant mass in each period are shown in Table 1.

Crotalaria biomass production was evaluated in 1 m^2 of an adjacent area, grown with *Crotalaria* with no urea spray. One week after ^{15}N-urea was sprayed, two plants per plot of the experiment were sampled, separated into shoot and root, and analyzed for total N and ^{15}N concentration. The *Crotalaria* plants were harvested at the flowering stage, 79 days after emergency. Shoot and root were analyzed separately. Roots were washed, dried under shade, and weighed. Plant shoot and root, as well as dead leaves collected from the ground were oven-dried (60°C) for the determination of dry mass, N content and ^{15}N abundance. Nitrogen content was determined by the micro-Kjeldahl digestion-distillation method (Bremner & Mulvaney, 1982) and ^{15}N by the mass spectrometry using the sampling preparation described by Trivelin et al. (1973). Plant chemical analysis for determination of macro- and micronutrients was performed according to Bataglia et al. (1983).

After harvesting the *Crotalaria* plants, soil was sampled at the 0-20 cm and 20-40 cm depths for fertility analysis. Two composit samples were assembled with the soil of the 8 plots treaded with ^{15}N-urea and the 4 control plots, respectively.

RESULTS AND DISCUSSION

The degree of labeling of the *Crotalaria* plants increased with time due to successive ^{15}N-urea applications. The plant samples collected 7 days after the first urea spray had concentrations of ^{15}N of 0.657 atoms % for shoot and 0.875 atoms % for root. The corresponding values for the plants at flowering, after three urea applications, were 2.412 and 1.644 atoms %

^{15}N, with dry matter yield of about 9.1 and 1 t/ha for shoot and root, respectively (Table 1). The results indicate that ^{15}N enrichment of the *Crotalaria* plants was efficient and even the roots reached a reasonable ^{15}N-labeling. Therefore, this field labeling technique seems to be adequate for studies on nitrogen dynamics using plant material that will be added to the soil and undergo isotopic dilution until it is analyzed in the subsequent crop.

Samples of *Crotalaria* taken 7 days after the first and the second urea application were thoroughly rinsed with distilled water before ^{15}N determinations. The results of ^{15}N concentration (data not shown) were similar to those obtained with plants that were only oven-dried, indicating that the ^{15}N was rapidly incorporated to the plants and could not be removed by the cleaning procedures used for plant analysis.

A lower labeling level (<2 ^{15}N atoms %) can negatively affect accurate determination in isotopic analyses. The dry plant material contained more than 190 kg ha^{-1} of N. These values can vary greatly. Muraoka et al. (2002) observed a variation from 149 to 362 kg ha^{-1} N, affected by the dry matter production but not by the N contents, which were similar.

Table 2 shows the data on yield and N concentration of the Crotalaria at the flowering stage, when it was cut to be used as green manure. That the dry matter yield of shoot and root as well as the N concentration of the plants sprayed with ^{15}N-urea were similar to those of the control plots, suggesting that the labeling technique was adequate for labeling the *Crotalaria* plants without changing the N content of the plant. Dead leaves that fell from the plants along the growing cycle represented a small part of the total dry matter produced, but were highly enriched with ^{15}N (Table 2).

The nutrient contents shown in Table 3 are similar to those found in field experiments with *Crotalaria* by Tanaka et al. (1992) and Muraoka et al. (2002), although Mn and Fe values are a little higher because the parent material of the Paleudalf presents high contents of those nutrients. The results also indicate that the foliar spray of small amounts of ^{15}N labelled urea did not change the macro and micronutrient contents of the plants.

Table 1 - Application dates of ^{15}N-containing urea. Characterization of *Crotalaria* shoot and root performed seven days after fertilizer application.

Applicatio Date	Fresh mass	Dry mass	N content		15N	Plant height
	kg ha^{-1}		g kg^{-1}	kg ha^{-1}	Atom %	m
	Shoot					
01/11/01	3055	638	47.0	29.9	0.657	0.45
01/31/01	19000	3560	15.0	53.4	1.596	1.75
02/15/01	22500	4703	43.0[1]	-	3.260	1.95
02/20/01[2]	33393	9150	21.4	195.8	2.412	2.00
	Root					
01/11/01	310	61	8.4	0.5	0.875	-
01/31/01	2300	463	9.7	4.5	1.332	-
02/15/01	2150	515	[1]-	-	-	-
02/20/01[2]	3532	1052	7.7	8.1	1.644	-

[1]Only leaves were analyzed for labeling control. [2]Harvest date.

Table 2 - Fresh and dry mass and N content of different *Crotalaria* plant parts at harvesting. Results are means of four replicates.

Plant part[1]	Treatment[2]	Fresh mass	Dry mass	15N	N content	
		kg ha^{-1}		atom %	g kg^{-1}	kg ha^{-1}
Shoot	T1	30625	9134	0.372	23.7	216.5
Dead leaf	T1	508	331	0.369	15.7	5.2
Root	T1	3007	885	0.367	9.0	7.9
Shoot	T2	35000	9366	2.136	22.0	206.3
Dead leaf	T2	594	303	2.050	13.9	4.2
Root	T2	3782	1140	1.494	7.5	8.6
Shoot	T3	31787	8933	2.687	20.7	184.9
Dead leaf	T3	554	298	2.292	14.6	4.3
Root	T3	3282	964	1.793	7.9	7.6

[1]Dead leaves collected on the ground. [2]T1= Control (*Crotalaria* with no ^{15}N). T2 and T3= treatments with ^{15}N-labeled *Crotalaria*.

Table 3 - Macro and micronutrient contents and C/N ration in different plant parts of *Crotalaria* sampled at harvesting.

Plant material[1]	Treatment[2]	K	P	Ca	Mg	B	Cu	Fe	Mn	Zn	C/N
		g kg^{-1}				mg kg^{1}					
Shoot	T1	7.2	2.1	9.2	5.0	36.2	10.8	527	69.8	22.7	31
Dead leaf	T1	2.3	1.5	40.9	14.5	58.2	13.6	4872	300.3	21.9	34
Root	T1	6.7	1.0	1.4	3.2	25.0	8.4	5619	30.5	12.1	52
Shoot	T2	7.8	1.9	8.3	4.2	41.5	8.2	832	43.0	18.2	27
Dead leaf	T2	1.8	1.1	32.6	12.2	54.2	11.4	7035	199.8	20.8	26
Root	T2	7.4	0.9	1.2	2.8	26.9	8.0	8737	34.0	11.7	55
Shoot	T3	6.2	1.8	7.0	4.3	39.3	8.6	935	41.8	17.4	23
Dead leaf	T3	1.8	1.4	33.4	13.3	50.7	13.1	8990	227.3	27.0	26
Root	T3	6.7	1.0	1.2	3.2	27.3	7.8	6215	30.0	11.5	58

[1]Dead leaves collected on the ground. [2]T1= Control (Crotalaria with no ^{15}N). T2 and T3= treatments with ^{15}N-labeled Crotalaria.

Table 4 - Analyses of soil from plots grown with *Crotalaria* and control plots kept uncultivated. Samples were taken at the 0-0.2 and 0.2-0.4 m depths.

Soil variable	Uncultivated soil		Soil with Crotalaria	
	0-0.2 m	0.2-0.4 m	0-0.2 m	0.2-0.4 m
pH	4.1	4.0	4.5	4.7
O.M. (g dm^{-3})	26	22	24	22
P (mg dm^{-3})	3	14	6	6
S (mg dm^{-3})	12	15	8	8
K (mmol$_c$ dm-3)	0.7	0.5	0.3	0.3
Ca (mmol$_c$ dm^{-3})	7	6	12	11
Mg(mmol$_c$ dm^{-3})	6	5	11	10
H + Al (mmol$_c$ dm^{-3})	50	68	36	31
Al (mmol$_c$ dm^{-3})	10	11	2	2
Base saturation (mmol$_c$ dm^{-3})	13.7	11.5	23.3	21.3
CEC (mmol$_c$ dm^{-3})	63.7	79.5	59.3	52.3
V %	22	14	39	41

CONCLUSION

The production of ^{15}N-labeled green manure without changing its chemical characteristics was successful. The procedure used for isotopic nitrogen labeling of *Crotalaria juncea* in the field was adequate because it was possible to produce large quantities of plant material with a degree of ^{15}N enrichment of both, shoot and root, satisfactory for their use in "*in loco*" studies on the fate of the N from green manure.

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