

## Responses of *Eucalyptus benthamii* seedlings to the application of the organic fertilizer Bacsol

Respuestas de las plántulas de *Eucalyptus benthamii* a la aplicación del fertilizante orgánico Bacsol

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### SUMMARY

*Eucalyptus benthamii* is a tree species commonly planted in subtropical areas, including the Southern Brazil, due to its tolerance to low temperatures and frosts. The success of *E. benthamii* plantation depends on the seedlings vigor during transplantation. We hypothesized that improvement of chemical and biological properties of growth substrate would produce more vigorous *E. benthamii* seedlings. Thus, we applied increasing doses (0, 0.5, 1.0, 1.5 and 2.0 g per seedling) of the organic fertilizer Bacsol, which carries a consortium of potential plant growth promoting microorganisms, on the substrate, and measured the growth and development of *E. benthamii* seedlings. Two experiments were performed in 2011 and 2012. The first experiment included measurements in four growth stages to estimate the relative growth rates. The second one ended at the time that seedlings reached standard heights for transplantation (about  $\pm$  20 cm). Measurements on plant height, stem diameter, shoot dry weight and relative growth rates indicated that the application of Bacsol improved *E. benthamii* seedlings vigor and decreased the time needed in nursery, from 150 days to 90 days. The best responses occurred at doses from 1.3 g to 1.5 g per seedling. The data demonstrated that application of Bacsol in substrate is a promising technology to increase *E. benthamii* seedlings vigor.

**Key words:** plant growth promoting microorganisms, efficiency of nutrient utilization, nursery, relative growth rates.

### RESUMEN

*Eucalyptus benthamii* es una especie arbórea comúnmente plantada en áreas subtropicales, incluyendo el sur de Brasil, debido a su tolerancia a bajas temperaturas y heladas. El éxito de su plantación depende del vigor de las plántulas durante el trasplante. Se planteó la hipótesis que la mejora de las propiedades químicas y biológicas del sustrato de crecimiento produciría plantas más vigorosas de *E. benthamii*. Por ello, en el sustrato se aplicaron dosis crecientes (0, 0,5, 1,0, 1,5 y 2,0 g por planta) del fertilizante orgánico Bacsol, que contiene un consorcio de potenciales microorganismos promotores del crecimiento vegetal y se midió el crecimiento de plantas de *E. benthamii*. Los experimentos fueron realizados en 2011 y 2012. El primero incluyó cuatro etapas de crecimiento para estimar tasas de crecimiento relativas. El segundo terminó cuando las plantas alcanzaron alturas estándar para trasplante ( $\approx$  20 cm). La altura de la planta, el diámetro del tallo, el peso seco de los brotes y las tasas de crecimiento relativo indicaron que Bacsol mejoró el vigor de las plantas y disminuyó el tiempo necesario en vivero, de 150 a 90 días. Las mejores respuestas ocurrieron con 1,3 a 1,5 g por planta. Los datos demostraron que la aplicación de Bacsol al sustrato es una tecnología prometedora para aumentar el vigor de las plantas de *E. benthamii*.

**Palabras clave:** microorganismos promotores del crecimiento de las plantas, eficiencia de la utilización de nutrientes, vivero, tasas de crecimiento relativas.

### INTRODUCTION

Brazil embraces about 7.75 million ha with planted forests and about 35 % of this area is devoted to the plantation of *Eucalyptus* genus. In Southern Brazil, the dominant tree species is the *Eucalyptus benthamii* Maiden et Camba-

ge due to its tolerance to low temperature and frosts (Kratz *et al.* 2013). Although *E. benthamii* is well adapted to climate conditions in the region, the success of plantations is limited by the vigor of seedlings transplanted to the field. Vigorous seedlings must have well developed roots, well-grown shoots and adequate nutritional status (Wendling

*et al.* 2001) and result from good management in sowing, growth and pre-acclimation (Wendling and Dutra 2010). Despite of the recent improvements in nursery activities, *E. benthamii* still takes more than 150 days to sufficiently grow to be transplanted to the field (Kratz *et al.* 2013). Large seedlings are obtained by adding high quantities of fertilizers to the substrate (Trindade *et al.* 2001, Wendling and Dutra 2010), but fertilizers alone do not always ensure vigorous seedlings since, in addition to abiotic factors, biological factors play a role in plant growth (Saharan and Nehra 2011). Therefore, in addition to the chemical amendment, biological aspects must be considered in the program of seedling production.

Several studies have shown benefits from (re)inoculating microorganisms isolated from the rhizosphere, with particular emphasis on rhizobacteria. Rhizobacteria have various metabolic mechanisms that stimulate plant growth, such as phytohormone production, biological nitrogen fixation, phosphate solubilization (Richardson *et al.* 2009) and systematic biological control of plant pathogens (Teixeira *et al.* 2007, Richardson *et al.* 2009). Several studies show plant responses due to the inoculation of individual rhizobacteria species. To date, nursery seedlings of *Picea glauca* (Moench) Voss inoculated with the rhizobacteria *Paenibacillus polymyxa* (Prazmowski 1880) Ash *et al.* 1994 and, as well as seedlings of *Pseudotsuga menziesii* (Mirb.) Franco (O'Neill *et al.* 1992), *Pinus taeda* Blanco, *Pinus elliottii* Engelm. (Enebak *et al.* 1998) and *Pinus contorta* Dougl. (Chanway and Holl 1991) inoculated with two bacteria (*P. polymyxa* and *Pseudomonas fluorescens* Migula) grew more than their counterpart non-inoculated seedlings. In addition, *in vitro* experiments involving a number of one-to-one combinations (*Eucalyptus cloeziana* F. Muell and *Eucalyptus globulus* Labill inoculated with *Pseudomonas* sp.; *Eucalyptus grandis* Hill ex Maiden inoculated with *Bacillus subtilis* (Ehrenberg) Cohn; *Eucalyptus urophylla* S. T. Blake inoculated with *Pseudomonas fulva* Iizuka *et* Komagata; and *Stenotrophomonas maltophilia* Palleroni *et* Bradbury inoculated with *B. subtilis*) confirmed that inoculation of seedlings with rhizobacteria promoted faster root development (Mafia *et al.* 2009). Furthermore, *E. grandis* and a clone *E. grandis* x *E. urophylla* inoculated with a peat formulation of *B. subtilis* had increased root development and resulted in superior plant growth (Zarpelon 2007). We hypothesized that improvement of biochemical properties of substrate growth would produce more vigorous seedlings. Thus, amendment of substrate with a consortium of microorganisms playing roles in several processes in the rhizosphere would also contribute to faster growth of inoculated seedlings in relation to non-inoculated seedlings.

The organic fertilizer Bacsol carries nutrients and several microorganisms with potential to promote plant growth. Microorganisms carried in Bacsol are latent, however they grow when they meet favorable conditions (adequate temperature, wet soil and plant exudate availability) for mul-

tiplication and activity. Bacsol has usually been applied to annual crops, nevertheless there is a potential of it stimulating tree seedling growth under nursery conditions. Indeed, application of Bacsol resulted in significant increase in plant growth of *Acacia mearnsii* de Wild. (Hoppe *et al.* 2004a), *Pinus elliottii* Engelm. (Hoppe *et al.* 2004a) and *Ilex paraguariensis* A. St.-Hil. (Hoppe *et al.* 2005). Therefore, we aimed at measuring the responses of *E. benthamii* to the application of Bacsol in the substrate. In addition, we aimed at determining the dose of application in nursery for the best plant growth response.

## METHODS

*Experiment setup and field conditions.* Two experiments were setup to evaluate the effects of Bacsol® (RSA Biotecnologia Agrícola, Barueri, São Paulo, Brazil) upon *E. benthamii*, at increasing solid doses: 0 (not inoculated), 0.5, 1.0, 1.5 and 2.0 g per seedling. Bacsol is an organic fertilizer composed by a mixture of soybean meal, rice straw and yeast extract functioning as a carrier of microorganism. The product Bacsol used in the experiments contained nine different species of potential plant growth promoting microorganisms: *B. subtilis*, *Bacillus megaterium* de Bary, *Bacillus natto* Sawamura, *Clostridium butyrycum* Prazmowski, *Nitrossomonas* spp., *Nitrobacter* spp., *P. fluorescens*, *Saccharomyces cerevisiae* Meyen ex E. C. Hansen and *Streptomyces* spp., containing, according to manufacture,  $1 \times 10^6$  cells of each microorganism per gram.

The first experiment was set in a randomized block design, with three blocks, five treatments (doses) and 240 seedlings per treatment. Plant height of seedlings was measured at regular intervals (60, 90 and 120 days in 2011, and 30, 60 and 90 days in 2012) and values were used to calculate the relative growth rates (RGR). The second experiment was set in a completely randomized design with 50 seedlings per treatment, determining shoot dry weight (SDW), plant height (PH), stem diameter (SD), nutrient concentrations (N, P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn) and their related parameters. Harvest time occurred on basis of standard height for seedling transplantation to the field (about 20 cm). In 2011, the final harvest occurred on the 150<sup>th</sup> day according to the height of control treatment. And in 2012, the final harvest occurred on the 100<sup>th</sup> according to the inoculated treatments, as the inoculated seedlings of 2011 grew too much for standard height.

Seedlings were formed from pelletized seeds sown in plastic tubes (53 cm<sup>3</sup>) filled with Carolina Soil® substrate, not sterilized, composed by pine bark and vermiculite (Carolina Soil do Brasil Ltda., Santa Cruz do Sul, Rio Grande do Sul, Brazil) mixed with the doses of the inoculant and standard fertilization used in the nursery.

At sowing, standard fertilization consisted of application of 3.7 mg of nitrogen, 46 mg of phosphorus, 7.5 mg of potassium, 31 mg of calcium, 13 mg of sulfur, 0.26 mg

of STAUBC® additive, 6.5 mg of magnesium, 0.093 mg of boron, 0.047 mg of copper, 0.28 mg of manganese, 9.37 mg of silicon and 0.51 mg of zinc fertilizer per seedling. Thirty-five days after sowing, seedlings were sprayed twice a week with foliar fertilizer, comprising mono ammonium phosphate (MAP 12-61-0) (630 g), calcium nitrate (320 g), potassium chloride (270 g), iron (191 g), boric acid (15 g) and vitamin complex (10 g) dissolved in 500 liters of water.

The experiments were performed under outdoor nursery field conditions in Guarapuava, PR, Brazil (S 25°19'35.55" and W 51°29'56.73"), with climate type Cfb, according to Köppen's Classification (Alvares *et al.* 2013). In 2011, temperatures averaged 13 °C, with 21 °C maximum and 6 °C minimum. And in 2012, 17 °C, with 26 °C maximum and 10 °C minimum.

Seedlings were wetted every day, twice a day, except when it was raining.

*Plant measurements.* Plant height (PH) was determined as the length from the base stem to the highest leaf tip. Stem diameter (SD) was the thickness of stem at 1.5 cm above the substrate surface. Shoot dry weight (SDW) was the weight of above ground biomass dried at 65 °C for three days. Measurements of plant height, stem diameter and shoot dry weight were used to calculate ratios of PH/SD and PH/SDW. Shoots were split into leaves and stems, grounded and submitted to dry digestion for determination of nutrient elements (P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn) (Martins and Reissmann 2007). The nitrogen content into leaves and stems was measured and determined with an Elementar Vario EL III (Elementar, Hanau, Germany) using 1 and 0.5 mg of ground and homogenized material of leaves and stems.

*Calculated indicators.* The efficiency of nutrient utilization (E), representing the efficiency of plant nutrients to produce plant biomass was estimated through the equation 1 (Siddiqi and Glass, 1981):

$$E = (SDW)^2/(Q) \quad [1]$$

Where: Q = nutrient content in that biomass (g), SDW = shoot dry weight (g).

The relative growth rate (RGR) was taken from the slope of the first-order regression estimated with shoot dry weight at harvesting times (60, 90, 120 and 150 days in 2011, and 30, 60, 90 and 120 days in 2012).

The accumulated shoot dry weight in 150 days in 2011 and 120 days in 2012 was adjusted into quadratic regressions as variables depending on the inoculant doses. The quadratic functions were differentiated to determine the maximal dose (g seedling<sup>-1</sup>). The maximal values of shoot dry weight were obtained after filling the original quadratic functions with the maximal doses of the derivative functions.

*Statistics.* Data were checked for normality and for variance of homogeneity and submitted to the analysis of variance (ANOVA). Test F revealed that inoculation resulted in a significant effect in all parameters, and for that, test Tukey was applied on the differences among averages.

## RESULTS

Measurements on plant height, stem diameter, shoot dry weight and shoot nutrient contents indicated that Bacsol promoted *E. benthamii* growth. All inoculated seedlings grew more and had faster development than that observed in non-inoculated seedlings (table 1). Regardless of doses or year, inoculation with the organic fertilizer resulted in increases of 56 % in shoot dry weight and 38 % in plant height contrasted with non-inoculated seedlings. These results implied that inoculation reduced the time in which seedlings needed to grow under nursery conditions. In fact, by analyzing the results of the 2011-experiment, we decided to harvest the 2012-experiment one month earlier since 120 days were enough for inoculated plants to reach standard height (20 cm).

Doses of Bacsol inoculant as low as 0.5 g per seedling were enough to make the relative growth rates (RGR) of inoculated seedlings to be significantly higher than that presented by non-inoculated seedlings (table 1).

Measurements on shoot nutrient contents aimed at verifying the effects of the biotechnological product on plant nutrition. There were no differences in the concentration of nutrients in the shoots of different treatments (data not shown), although differences were found in the way the nutrients were used to build up biomass (table 1). The efficiency of nutrient utilization (E), calculated as the ratio of biomass produced by the amount of nutrient absorbed by plants, indicated that Bacsol did not increase the total of nutrients taken up by plants; rather, it improved their utilization. Higher values of E were observed in doses 0.5 and 1.0 g per seedling (table 2). However, at the higher tested dose (*i.e.* 2.0 g per seedling), the value of E decreased significantly compared with lower doses (table 2). Therefore, it appeared that seedlings responded positively to the inoculant in intermediary doses, nevertheless after that, they were negatively affected. Our data set does not allow us to understand the reasons for such response patterns, but it indicates that, in practical terms, there is an ideal dose of inoculant for the best results.

Modelling of the parameters of seedling growth indicated that doses between 1.3 g (in 2011) and 1.5 g (in 2012) of inoculant per seedling resulted in the best growth responses (table 3), however higher doses resulted in negative responses.

**Table 1.** Responses of *Eucalyptus benthamii* seedlings to inoculation of Bacsol® doses as measured by shoot dry weight (SDW), plant height (PH), stem diameter (SD), the ratio of PH/SD, the ratio of PH/SDW and relative growth rate (RGR).\*

Respuesta de plántulas de *Eucalyptus benthamii* a la inoculación de dosis de Bacsol® determinadas por peso de biomasa seca aérea (SDW), altura (PH), diámetro del tallo (SD), relación PH/SD, relación PH/SDW y tasa relativa de crecimiento (RGR).

Treatment	SDW (g plant <sup>-1</sup> )	PH (cm)	SD (cm)	PH/SD (cm/cm)	PH/SDW (cm/g plant <sup>-1</sup> )	RGR (cm d <sup>-1</sup> )
2011						
Control	0.6 d	27.5 d	2.9 a	11.2 c	50.7 a	9.2 b
0.5 g BACSOL	1.1 c	37.7 c	2.8 a	13.5 b	36.2 b	13.4 ab
1.0 g BACSOL	1.4 b	43.3 b	3.0 a	14.4 b	33.0 bc	14.9 a
1.5 g BACSOL	1.5 a	52.6 a	3.1 a	17.1 a	35.4 b	15.6 a
2.0 g BACSOL	1.5 ab	42.8 b	3.1 a	13.7 b	29.6 c	17.5 a
CV (%)	24.4	5.9	48.9	12.5	26.3	10.8
ANOVA <i>P</i> -value	***	***	Ns	***	***	***
2012						
Control	0.4 d	21.8 d	0.7 d	30.6 b	57.1 a	9.2 b
0.5 g BACSOL	0.8 c	31.6 c	0.8 c	38.7 a	40.0 b	14.3 a
1.0 g BACSOL	1.1 a	37.3 ab	1.0 ab	38.4 a	38.6 b	15.4 a
1.5 g BACSOL	1.0 ab	38.3 a	1.0 a	37.9 a	40.2 b	13.4 ab
2.0 g BACSOL	0.9 bc	35.2 b	0.9 b	37.4 a	42.5 b	13.0 ab
CV (%)	34.1	12.2	11.7	14.2	30.3	12.3
ANOVA <i>P</i> -value	***	***	***	***	***	***

\* BACSOL inoculant ensured an individual concentration of  $1 \times 10^6$  cells g<sup>-1</sup> of the following microorganisms: *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus natto*, *Clostridium butyricum*, *Nitrossomonas* spp., *Nitrobacter* spp., *Pseudomonas fluorescens*, *Saccharomyces cerevisiae* and *Streptomyces* spp. Values of SDW, PH, SD, PH/SD and PH/SDW represent the average of measurements taken in 50 seedlings when they were 150 days-old in 2011 and 100 days-old in 2012. RGR were calculated with measurements of SDW taken when seedlings were 60, 90 and 120 days-old in 2011, and 30, 60 and 90 in 2012. Differences of means within the columns are expressed with different letters indicated by the Tukey test ( $P \leq 0.001$ ).

**Table 2.** Responses of *Eucalyptus benthamii* seedlings to inoculation of Bacsol®\* doses as measured by the efficiency of nutrient utilization (E) for leaves and stems contents of N, P, K, Na, Ca, Mg, Fe, Cu, Mn and Zn.\*

Respuesta de plántulas de *Eucalyptus benthamii* a la inoculación de dosis de Bacsol® determinadas por la eficiencia de utilización (E) para los elementos N, P, K, Na, Ca, Mg, Fe, Cu, Mn y Zn de hojas y tallos.

Treatment	E (g <sup>2</sup> /g)									
	N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Leaves										
Control	34.4 d	148.6 b	34.0 b	63.1 c	75.5 b	231.8 d	1.8 d	65.0 bc	0.5 c	12.1 d
0.5 g BACSOL	55.7 a	352.6 a	56.4 a	122.0 b	135.7 a	465.2 c	4.8 c	83.1 b	1.2 b	22.3 c
1.0 g BACSOL	50.9 ab	365.5 a	17.3 c	163.5 a	163.1 a	700.3 a	8.7 a	117.8 a	1.9 a	40.0 a
1.5 g BACSOL	45.8 bc	348.1 a	19.8 c	125.8 b	152.2 a	598.9 ab	6.6 b	77.8 b	1.8 a	31.1 b
2.0 g BACSOL	40.7 cd	312.6 a	14.2 c	162.0 a	150.5 a	566.5 bc	4.5 c	54.6 c	1.8 a	28.6 bc
CV (%)	35.8	39.8	49.6	40.5	39.5	42.7	48.7	43.3	39.4	45.9
Stems										
Control	28.8 c	24.2 c	16.4 b	21.6 c	120.1 b	126.8 c	1.7 b	4.2 b	0.8 d	3.4 d
0.5 g BACSOL	44.6 ab	66.9 b	21.1 a	48.3 b	185.8 a	301.8 b	3.5 a	16.5 a	1.9 c	10.1 b
1.0 g BACSOL	50.6 a	103.6 a	7.5 c	65.1 a	167.1 a	451.3 a	4.6 a	15.6 a	3.5 a	13.2 a
1.5 g BACSOL	48.3 a	108.3 a	7.5 c	49.5 b	158.5 a	347.5 b	3.7 a	14.3 a	2.8 b	9.2 bc
2.0 g BACSOL	37.5 bc	79.8 b	5.1 c	44.0 b	108.8 b	313.3 b	4.0 a	4.4 b	2.4 bc	7.5 c
CV (%)	35.8	43.8	53.9	43.5	46.0	45.5	69.8	51.6	56.4	51.6

\* BACSOL inoculant ensured an individual concentration of  $1 \times 10^6$  cells g<sup>-1</sup> of the following microorganisms: *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus natto*, *Clostridium butyricum*, *Nitrossomonas* spp., *Nitrobacter* spp., *Pseudomonas fluorescens*, *Saccharomyces cerevisiae* and *Streptomyces* spp. Values of E for all nutrients represent the average of measurements taken in 10 seedlings when they were 150 days-old in 2011 and 100 days-old in 2012. E was calculated as a ratio of the biomass (g) produced per mg of nutrient accumulated in the biomass. ANOVA  $P \leq 0.001$  for all variables. Differences of means within the columns are expressed with different letters indicated by the Tukey test ( $P \leq 0.001$ ).



**Table 3.** Maximum responses of *Eucalyptus benthamii* seedlings to different doses of Bacsol®\*.  
 Máxima respuesta de plántulas de *Eucalyptus benthamii* a diferentes dosis de Bacsol®\*.

Parameter	2011			2012		
	Dose BACSOL (g)	Maximum value	Adjusted R <sup>2</sup>	Dose BACSOL (g)	Maximum value	Adjusted R <sup>2</sup>
SDW (g plant <sup>-1</sup> )	1.6	1.6	0.99	1.3	1.1	0.99
PH (cm)	1.4	48.0	0.89	1.4	38.5	0.99
SD (cm)	1.8	3.1	0.82	1.5	1.0	0.95
PH/SD (cm/cm)	1.3	15.6	0.78	1.3	39.4	0.82
PH/SDW (cm/g plant <sup>-1</sup> )	1.6	30.9	0.85	1.2	37.0	0.89
Average dose	1.5	-	-	1.3	-	-

\* Values are related to the maximum results of shoot dry weight (SDW), plant height (PH), stem diameter (SD), the ratio of PH/SD and the ratio of PH/SDW. The adjusted R<sup>2</sup> refers to goodness of quadratic regressions.

## DISCUSSION

Our results demonstrated that application of Bacsol in the substrate increased the rates of plant growth of *E. benthamii* (table 1) in such a way that seedlings were ready for transplantation 30 days earlier than usual time. In addition, the application of Bacsol improved the ratio PH/SDW; that is, resulted in more abundant shoot dry weight regarding plant height (lower PH/SDW), increasing the chances of seedling survival after transplantation to the field (Gomes *et al.* 2002). Therefore, the experiments demonstrated that the application of Bacsol is a prospective alternative to improve the system of seedling production.

We mentioned earlier that most experiments for increasing plant growth by inoculating potential plant growth promoting microorganisms have included only one or two species (mostly bacteria). These studies have produced controversial results, with positive, neutral or even negative results. In the case of *Eucalyptus* spp., for example, inoculation of *E. benthamii* with bacteria isolated from the rhizoplane of *E. benthamii* and *Pinus* sp. (Carmo 2013), and *E. grandis* x *E. urophylla* with *B. subtilis* (Paz *et al.* 2012) did not stimulate plant growth when compared to non-inoculated seedlings. On the other hand, inoculation of *E. cloeziana* and *E. grandis* with *Pseudomonas* sp., *E. globulus* with *B. subtilis* and *E. urophylla* with *B. subtilis*, *P. fulva*, *Pseudomonas* sp. and *S. maltophilia* stimulated germination and seedling growth (Mafia *et al.* 2009). Likewise, other rhizobacteria, such as *B. subtilis*, *Frateria aurantia* Swings *et al.*, *Pseudomonas aeruginosa* (Schroeter) Migula, *P. fulva*, *S. maltophilia* (Teixeira *et al.* 2007), *Bacillus firmus* Bredemann *et Werner*, *Bacillus mycoides* Flügge, *Bacillus stearothermophilus* Donk, *B. subtilis/amyloliquefaciens*, *Bacillus circulans* Jordan, *Brevibacillus brevis* Migula, *Paenibacillus lautus* (Nakamura) Heyndrickx *et al.* and *S. maltophilia* (Díaz *et al.* 2012) promoted better growth and development of the eucalypts root system.

An explanation for controversial results in literature is the fact that authors supposed that plants would respond to a singular inoculant strain, whereas, plants in natural ecosystems rely on complex and diverse microbial community. In our experiments, we applied a consortium of microorganisms (*B. subtilis*, *B. megaterium*, *B. natto*, *C. butyricum*, *Nitrossomonas* spp., *Nitrobacter* spp., *P. fluorescens*, *S. cerevisiae* and *Streptomyces* spp.) that potentially stimulate plant growth through synergistic and complementary mechanisms. To date, the effects of *B. subtilis* and *P. fluorescens* may be related to biological control of root pathogens (*e.g.* Paz *et al.* 2012, Bhardwaj *et al.* 2014), while *B. megaterium* and *C. butyricum* have a role in solubilizing soil phosphate (*e.g.* Bhardwaj *et al.* 2014), *Nitrossomonas* spp. and *Nitrobacter* spp. act as nitrifying bacteria in the soil nitrogen cycle (*e.g.* Ibiene *et al.* 2012) and, *S. cerevisiae* and *Streptomyces* spp. produce antimicrobial factors that probably regulate microorganisms by antibiosis (*e.g.* Agamy *et al.* 2013). In addition, these microorganisms may produce hormones (Bhardwaj *et al.* 2014). Our data does not allow splitting the different effects of each microorganism, although, the results indicate that the consortium of microorganisms contributed to larger growth and better development of inoculated seedlings in relation to non-inoculated seedlings of eucalyptus. It suggested that a consortium of microorganisms may aggregate several microbial functions and promote plant growth and seedling vigor in a more synergistic way.

A dose of 0.5 g of Bacsol per seedling was enough to increase growth and the responses increased up to 1.5 g per seedling. However, doses larger than 1.5 g per seedling were less beneficial. It is possible that, in high doses, microorganisms are competing for the same nutrient pools in the substrate (Kuzyakov and Xu 2013). Our modeling revealed that the best dose for maximum results were doses varying from 1.3 to 1.5 g of inoculant per seedling.

In conclusion, our experiments demonstrated that inoculation of *E. benthamii* with Bacsol, containing a consor-

tium of potential plant growth promoting microorganisms, resulted in bigger seedlings at shorter periods. Therefore, the experiments pointed out that inoculation is a prospective alternative to reduce costs and time of *E. benthamii* seedling production. Further studies are required to confirm whether seedlings treated with Bacsol would grow better in the field after transplantation.

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