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Selection of rhizobacteria for pre-emergence control of wild poinsettia, horseweed and sourgrass

Seleção de rizobactérias para o controle em pré-emergência de leiteiro, buva e capim-amargoso

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Abstract

This study aimed to select rhizobacteria isolates for pre-emergence control of three glyphosate-resistant species: horseweed (*Conyza sumatrensis*), sourgrass (*Digitaria insularis*), and wild poinsettia (*Euphorbia heterophylla*). Nineteen strains of *Bacillus* spp. and thirty-four strains of *Pseudomonas* spp. were isolated from soil samples from the states of Minas Gerais, São Paulo, Paraná, and Santa Catarina. These strains were evaluated *in vitro* and under greenhouse conditions for inhibition of seed germination and growth of target weeds. Several strains of *Bacillus* spp. and *Pseudomonas* spp. inhibited the germination and radicle growth of the three target species in Petri dishes containing agar-water medium with the respective bacterial isolates, separately. However, *Bacillus* spp. strains A1B1, A1B3, A1B4, A2B2, A2B3, A10B1, A10B5, A11B1, and A13B3 were the only ones to inhibit germination of horseweed seeds in greenhouse soil. The soil methodology (greenhouse) was more effective in the selection of strains than the *in vitro* methodology for not overestimating the bacterium-host interaction and for simulating better field conditions. Promising *Bacillus* spp. strains must be identified at the species level and characterized for metabolite production, plant growth regulation, root colonization, and effect on cultivated plants.

Additional keywords: Biological control; *Conyza sumatrensis; Digitaria insularis; Euphorbia heterophylla*.

Resumo

O objetivo deste trabalho foi selecionar isolados de rizobactérias para o controle em pré-emergência de três espécies que apresentam resistência ao glyphosate: buva (*Conyza sumatrensis*), capim-amargoso (*Digitaria insularis*) e leiteiro (*Euphorbia heterophylla*). Foram isolados 19 estirpes de *Bacillus* spp. e 34 de *Pseudomonas* spp. de amostras de solos de localidades, nos Estados de Minas Gerais, São Paulo, Paraná e Santa Catarina, e avaliados quanto à inibição da germinação de sementes e ao crescimento das plantas daninhas-alvo *in vitro* e em casa de vegetação. Vários isolados de *Bacillus* spp. e *Pseudomonas* spp. inibiram a germinação e o crescimento de radículas das três espécies-alvo em placas de Petri contendo o meio constituído por ágar-água com os respectivos isolados bacterianos, separadamente; contudo, somente os isolados de *Bacillus* spp. A1B1, A1B3, A1B4, A2B2, A2B3, A10B1, A10B5 A11B1 e A13B3 inibiram a germinação das sementes de buva em solo, em casa de vegetação. A metodologia em solo (casa de vegetação) foi mais eficaz para a seleção dos isolados do que a metodologia *in vitro* por não superestimar a interação entre a bactéria e o hospedeiro, e por simular melhores condições de campo. Os isolados de *Bacillus* spp. promissores deverão ser identificados a nível de espécie, caracterizados quanto à produção de metabólitos, reguladores de crescimento de plantas, colonização de radicular e efeito em plantas cultivadas.

Palavras-chave adicionais: Controle biológico; *Conyza sumatrensis; Digitaria insularis; Euphorbia heterophylla*.

Introduction

Weeds represent one of the main limiting factors for agricultural productivity worldwide due to their multiple deleterious effect on crops, crop interference, contamination of the product harvested with seeds and other plant parts, and increased moisture content of the product harvested, hindering its processing, preservation, and consequently reducing its value (Oliveira et al., 2011).

Chemical weed control is currently the most used method, given its low cost and high agronomic efficiency (Oliveira et al., 2011). The glyphosate molecule is the most widely used for this purpose and the adoption of crops resistant to this herbicide along with the end of its patent life in the year 2000, favored its wide acceptability and use (Duke & Powles, 2008). However, continuous use of this herbicide resulted in the selection of resistant weed biotypes in Brazil, such as horseweed (*Conyza sumatrensis* (Retz. E.Walker), ryegrass (*Lolium multiflorum* Lam.), sourgrass (*Digitaria insularis* (L.) Mez. ex. Ekman), windmill grass (*Chloris polydactyla* (L.) Sw.) (Barroso & Toledo, 2014 I, II, and III; Christoffoleti & Nicolai, 2016), and wild poinsettia (*Euphorbia heterophylla* L.) (Prigol et al., 2014).

Therefore, it is necessary new methods for controlling these species. Among the most attractive possibilities is the biological control, which consists in the suppression or stabilization of weed populations at subeconomic levels using natural enemies (Flores-Vargas & O'Hara, 2006).

Biological control based on the use of deleterious bacteria in plants, known as Deleterious Rhizobacteria (DRB), has been studied to aid weed management (Kennedy et al., 2001; Patil, 2014).

The rapid growth of bacterial isolates and, consequently, their greater facility for large-scale production can potentially make DRBs outstand fungi as bioherbicides (Li et al., 2003). In addition, nonphytopathogenic bacteria have been known to reduce seed germination, inhibit seedling growth, and decrease root growth and elongation by producing phytotoxins and plant regulators such as indole-3-acetic acid (IAA) (Kennedy et al., 2001).

The selection of rhizobacteria that can inhibit germination and/or plant growth has a relevant advantage due to the low possibility of resistance development (Flores-Vargas & O'Hara, 2006), further contributing with the environment and providing sustainable weed management.

Thus, this study selected rhizobacteria isolates for pre-emergence control of horseweed (*Conyza sumatrensis*), sourgrass (*Digitaria insularis*), and wild poinsettia (*Euphorbia heterophylla*). These species are considered of great importance for the Brazilian agriculture with reported cases of resistance to glyphosate and other herbicide molecules such as ALS and Protox inhibitors (Trezzi et al., 2006; Santos et al., 2014).

Materials and methods

Isolation of rhizobacteria from soil: *Bacillus* spp. and *Pseudomonas* spp.

Bacterial strains were isolated from 20 soil samples collected in different landscapes in four Brazilian states, as shown in Table 1. The sampling criterion chosen for the study was random sampling in agricultural production areas and native forests (Table 1). Approximately 100 grams of soil were collected in the 0-10 cm soil layer in each sampled site, being then placed in paper bags, stored in a thermal box, and sent to the laboratory for the isolation of rhizobacteria.

From the samples collected, specific laboratory methods were used to isolate bacteria from the genera *Bacillus* and *Pseudomonas*. The isolation of bacteria belonging to the genus *Bacillus* followed the methodology described by the World Organization (1985). To that end, one gram of soil was diluted in 10 mL of sterile saline solution (0.01 mM $CaCO_3$ $7H_2O$; 0.08 mM $MgSO_4.7H_2O$; 0.006 mM $FeSO_4.7H_2O$; 0.07 mM $MnSO_4.7H_2O$; 0.006 mM $ZnSO_4.7H_2O$; pH 7.0) in a 15 mL microtube, being then homogenized in vortex for one minute. Subsequently, a 1 mL aliquot was removed for pasteurization (12 minutes at 80 °C in a water bath – microprocessor controlled model Q215M - QUIMIS®, and subsequent thermal shock on crushed ice for 5 minutes), aiming at the selection of sporulating bacteria. Thereafter, the suspension was diluted four times, reaching a concentration of 10^{-4}. Then, 100 µL of the suspension was applied in a Petri dish with Tryptone Soybean Agar (TSA) medium, followed by incubation in a BOD chamber at 28 °C for 24 hours (Alfenas & Mafia, 2007). Colonies were individualized by morphological characteristics and maintained in Petri dishes containing the same medium mentioned above.

For the isolation of *Pseudomonas*, 10 g of soil from each sample was added in a 250 mL Erlenmeyer flask with 95 mL of sterile phosphate buffered saline (PBS, 10 mM K_2PO_4-KH_2PO_4, 0.14 M NaCl; pH 7.2), being stirred for 30 min at 200 rpm on a rotary shaker. Thereafter, 10^{-1} of the suspension was diluted in PBS and plated on King's B agar medium (King et al., 1954) supplemented with 75 mg L^{-1} cycloheximide to prevent fungal contamination, and with 75 mg L^{-1} penicillin and 45 mg L^{-1} novobiocin to prevent the growth of other bacteria. The plates were incubated at 28 °C for three days. The representative colonies were selected and pricked out through King's B agar streaks to obtain pure colonies (Tuite, 1969). Fluorescent pigment production was detected by exposing bacterial colonies to ultraviolet light (<260 nm wavelength). The isolates obtained were kept at 4 ºC in a refrigerator, being preserved by the mineral oil conservation technique (Alfenas & Mafia, 2007).

Table 1 - Description of soil samples collected for the rhizobacteria isolation to control of wild poinsettia (*Euphorbia heterophylla*), sourgrass (*Digitaria insularis*) and horseweed (*Conyza sumatrensis*) in pre-emergence.

ID	City	State	Latitude (S)	Longitude (W)	Collection date	Landscape	NBI	NPI
A1	São João da Mata	Minas Gerais	21°56'24"	46°00'36"	06/07/2016	NF	3	2
A2	Palmeira	Paraná	25°25'21.23"	49°59'59.99"	06/15/2016	Wheat	-	2
A3	Lagora Formosa	Minas Gerais	18°53'50.759"	46°31'46.92"	10/18//2016	Bean	-	7
A4	Alfredo Wagner	Santa Catarina	27°41'19.28	49°19'41"53	06/15//2016	NF	3	1
A5	São Joaquim da Barra	São Paulo	20°32.4'830"	47°51.1'950"	10/20//2016	Sugar cane	-	-
A6	Serra Negra	São Paulo	22°35.08'50"	46°47.2'16.0"	10/20//2016	Sugar cane	-	-
A7	Lagoa Formosa	Minas Gerais	18º49'29.788"	46°26'28.835"	10/25//2016	NF	-	1
A8	Bebedouro	São Paulo	20°14'53.174"	49°17'50.701"	07/18//2016	Citrus	-	5
A9	Bebedouro	São Paulo	20°22'0.214"	49°9'53.057"	07/18//2016	Citrus	3	3
A10	Icém	São Paulo	21°3'27.436"	48°28'16.669"	07/18//2016	Sugar cane	3	2
A11	Angatuba	São Paulo	23°30'37"	48°34'32.75"	07/20//2016	Corn	-	4
A12	Patos de Minas	Minas Gerais	18°31'02.0"	46°26'18.8"	09/27//2016	NF	3	-
A13	Lagoa Formosa	Minas Gerais	18°49'58.5"	46°26'32.3"	10/25//2016	Coffee	-	1
A14	Coromandel	Minas Gerais	18°28'51.182"	46°58'28.09"	01/23//2017	NF	-	2
A15	Coromandel	Minas Gerais	18°30'33.052"	46°57'27.846"	01/23//2017	NF	-	-
A16	Coromandel	Minas Gerais	18°40'12.997"	46°50'6.518"	01/23//2017	NF	-	1
A17	Coromandel	Minas Gerais	18°39'1.832"	46°42'2.484"	01/23//2017	NF	-	-
A18	Presidente Olegário	Minas Gerais	18°31.2'63"	45°97.4'15.1"	01/24//2017	NF	-	1
A19	Presidente Olegário	Minas Gerais	18°33'39.3"	46°01.0'32.4"	01/25//2017	NF	3	1
A20	João Pinheiro	Minas Gerais	18°53.5'71.9"	46°15.6'29.4"	01/24/2017	NF	-	-
Total strains per group of bacteria							19	33

ID: Soil sample identification; NF: Native forest; NBI: number of *Bacillus* spp. isolates; NPI: number of *Pseudomonas* spp isolates.

Evaluation of the *in vitro* antagonistic potential of rhizobacteria against target weeds

Cultures of each *Bacillus* spp. strains were produced in liquid Luria-Bertani medium (Alfenas & Mafia, 2007) at 25 °C for five days, in the absence of light, in 250 mL Erlenmeyer flasks under static conditions (without agitation). The selected isolates of *Pseudomonas* spp. were fermented in half-strength King's B medium (half the nutrients per liter) at 29 ºC, under 120 rpm rotary shaking in 250 ml Erlenmeyer flasks for 72 hours.

The concentration of bacterial strains was determined by the serial dilution technique in King's B medium for *Pseudomonas* spp. and TSA medium for *Bacillus* spp. Concentrations ranged from 10^4 to 10^5 CFU mL^{-1} for *Bacillus* spp., and from 10^8 to 10^9 CFU mL^{-1} for *Pseudomonas* spp.

Weed seeds, purchased from the company Agro Cosmos - Pesquisa e Plantas Daninhas, Shokucho do Brasil Ltda., were surface sterilized by immersion in 1% sodium hypochlorite solution (NaOCl) for three minutes. The seeds were then washed three times in sterile distilled water and transferred to sterile filter paper. A volume of 1 mL of bacterial suspension of each isolate was transferred to the surface of the agar-water medium in Petri dishes of 9 cm diameter and spread with the aid of a Drigalski loop. Fifteen seeds of each weed species were placed separately in each Petri dish and incubated in BOD chamber, as shown in Table 2. The control consisted of adding 1 mL of sterile water per Petri dish, followed by the distribution of 15 seeds of each weed species per plate, separately. Each bacterial strain was tested in five replicates, each replicate represented by a Petri dish with 15 seeds in a Completely Randomized Design (CRD). Germination percentage and root length (cm) were evaluated at seven days for wild poinsettia and 15 days for sourgrass and horseweed (Table 2). The data were submitted to Analysis of Variance and the means were grouped according to the Scott-Knott Test, both with significance of $p<0.05$.

Table 2 - Temperature (Temp.), photoperiod (Photop.) and incubation period (IP) in the laboratory experiment of rhizobacteria for control of wild poinsettia (*Euphorbia heterophylla*), sourgrass (*Digitaria insularis*) and horseweed (*Conyza sumatrensis*).

Species of weed	Temp. (°C)	Photop. (h)	IP (day)	References of growth conditions
E. heterophylla	25	12	7	Suda & Pereira, 1997
D. insularis	22	12	15	Mondo et al., 2010
C. sumatrensis	22	12	15	Nandula et al., 2006

Evaluation of the antagonistic potential of rhizobacteria against target weeds under greenhouse conditions

For the selection of bacterial strains under greenhouse conditions, the cultures of each of the isolates of *Pseudomonas* spp. were produced as described in the previous experiment. The production of *Bacillus* spp. was modified due to the problem of low final concentration of isolates (10^4 and 10^5 CFU mL^{-1}) verified in the production for the *in vitro* experiments. In this way, we opted for the growth of isolates under agitation (120 rpm), obtaining variations in the concentrations of isolates between 10^8 and 10^9 CFU mL^{-1} (closer to the industrial reality of biological control agents). Concentrations ranged from 10^8 to 10^9 CFU mL^{-1} for *Pseudomonas* spp. strains.

Fifteen seeds of *E. heterophylla*, 0.01 g of *C. sumatrensis* seeds, and 0.03 g of *D. insularis* seeds were sown separately into 250 mL styrofoam containers (8 cm diameter and 10 cm height) containing a sterilized soil/sand mixture (1:1) and covered with a 0.5 cm layer of Carolina®/coarse sand (2:1) substrate mixture. Soon after sowing, a 1.0 x 10^7 suspension of each isolate was prepared and sprayed onto the surface of the containers with an airbrush (SagymaPro, model SW-130K) with syrup volume corresponding to 200 L ha^{-1}. The containers were stored in the greenhouse and 2-mm irrigation was performed by sprinkler irrigation immediately after spraying the isolates. Containers were irrigated manually twice a day, and the temperature during the conduction of the assays varied from 14 to 29 °C during the 14 days for *E. heterophylla* and *D. insularis*, and during the 21 days for *C. sumatrensis*.

The number of germinated plants was evaluated at the end of the assays. Plants were harvested and shoot and root length (cm) were measured using a digital caliper (ABSOLUTE - Mitutoyo Sul Americana Ltda.). The roots and shoots of plants were dried at 60 °C for 24 hours in a MA035 (Marconi®) sterilization and drying oven with forced air circulation being then weighed using a precision scale.

The following parameters were evaluated: SG (% seed germination), RDL (radicle length), RL (root length), SL (shoot length), TL (total length), SDW (shoot dry weight), RDW (root dry weight), and TDW (total dry weight).

Results and discussion

In the 20 soil samples, a total of 41 isolates of *Bacillus* spp. and 33 isolates of *Pseudomonas* spp. was obtained. Among the isolates of *Bacillus* spp., 22 were discarded because they presented crystals characteristic of *Bacillus thuringiensis* (Berliner, 1915), which were not in the scope of the present study. Each of the samples A1, A4, A9, A10, A12, and A19 presented three isolates of the genus *Bacillus* without presence of crystals. For *Pseudomonas* isolates, the samples with the highest populations were: A3, col-

lected in a bean cultivation area in Lagoa Formosa-MG; A8, from a citrus orchard in Bebedouro-SP; and A14, from a maize farm in Angatuba-SP (Table 1).

In the *in vitro* assay, six isolates of *Bacillus* spp. significantly inhibited the germination of *E. heterophylla* seeds, namely: A1B1, A1B4, A2B1, A2B2, A11B2, and A13B3 (Table 3). Of these isolates, four reduced root growth (A1B1, A2B1, A2B2, A13B3), as well as a further ten isolates of *Bacillus* spp. (A1B3, A2B1, A2B3, A5B3, A10B1, A10B4, A10B5, A11B1, A13B2, A13B3), as shown in Table 3.

In greenhouse assays, none of the *Bacillus* spp. isolates was able to reduce the germination and root and shoot growth of *E. heterophylla* plants (Table 3). On the other hand, four isolates promoted increased SDW of *E. heterophylla* (A1B3, A10B4, A11B2, and A13B2). These same isolates also promoted increased TDW, as observed for two other isolates (A2B1 and A10B5) (Table 3). A similar result was found by Li & Kremer (2006), with reduction of SDW (shoot dry weight) and no reduction of RDW (root dry weight) with the application of bacteria *Aeromonas hydrophila* (Chester, 1901) Stainer, 1943, isolate TMR13, and *Pseudomonas aureofaciens* (Kluyver, 1956), isolate TMH16, in *Convolvulus arvensis* L. plants. Isolate A11B2, which increased SDW under greenhouse conditions, reduced the *in vitro* germination percentage.

All these isolates that promoted plant growth under greenhouse conditions are among those that reduced *in vitro* germination or root growth. This effect is believed to be directly related to the suspension rate used in the assays, and may relate both to the concentration of bacterial cells per seed and to the concentration of regulators such as indole-3-acetic acid (IAA), since the fermentation methodology and CFU values in each assay were different. The IAA seems to play a double role according to its concentration (Chauan et al., 2009). High concentrations of IAA secreted by bacteria inhibit the germination of weed seeds (Tabatabaei et al., 2016), and are also produced by most plant growth-promoting bacteria (PGPB), being considered one of the most effective mechanisms already elucidated (Lin et al., 2012). At high concentrations, the IAA can also reduce plant root growth by its relationship with ethylene synthesis. This hormone can stimulate the transcription of 1-aminocyclopropane-1--carboxylic acid (ACC) synthase, which is the enzyme responsible for oxidizing ACC during ethylene biosynthesis (Ju & Chang, 2015). This may be the reason why the root development of *E. heterophylla* decreased significantly after contact with some *Bacillus* spp. isolates in the *in vitro* experiment.

Table 3 - Effect of *Bacillus* spp. strains on wild poinsettia (*Euphorbia heterophylla*) control in laboratory and greenhouse conditions.

Strains	Laboratory experiment		Greenhouse experiment						
	SG (%)	RDL (cm)	SG (%)	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
Control	18.6 a	5.2 a	65.3 a	11.1 a	5.0 a	16.2 a	8.7 c	4.7 a	13.4 b
A1B1	5.3 b	1.1 c	50.2 a	10.6 a	4.5 a	15.2 a	7.7 c	4.4 a	12.1 b
A1B3	16.6 a	2.7 b	58.6 a	11.6 a	4.9 a	16.4 a	12.9 a	4.7 a	18.0 a
A1B4	8.9 b	3.5 a	64.0 a	11.5 a	4.9 a	16.4 a	8.6 c	4.3 a	13.0 b
A1B5	14.0 a	3.6 a	85.9 a	9.9 a	4.8 a	14.9 a	8.3 c	4.0 a	12.2 b
A2B1	6.6 b	1.6 c	68.7 a	12.9 a	4.9 a	17.4 a	9.7 c	5.2 a	14.9 a
A2B2	6.6 b	1.7 c	58.0 a	12.0 a	5.3 a	17.3 a	9.4 c	4.8 a	14.2 b
A2B3	14.6 a	1.5 c	82.6 a	10.4 a	4.9 a	15.4 a	7.8 c	4.0 a	11.9 b
A5B1	12.9 a	3.1 a	71.3 a	9.8 a	5.0 a	14.9 a	8.3 c	4.2 a	12.5 b
A5B3	12.5 a	2.5 b	67.8 a	11.9 a	5.0 a	16.9 a	8.6 c	4.5 a	13.2 b
A5B4	12.1 a	3.8 a	70.1 a	11.7 a	5.0 a	16.7 a	9.0 c	4.1 a	12.9 b
A10B1	13.4 a	2.4 b	68.0 a	11.2 a	5.1 a	16.4 a	8.7 c	5.5 a	14.3 b
A10B4	13.8 a	2.5 b	65.3 a	10.3 a	6.2 a	17.2 a	10.3 b	5.3 a	15.6 a
A10B5	13.8 a	2.9 b	67.8 a	12.7 a	5.2 a	17.9 a	9.5 c	5.2 a	14.8 a
A11B1	17.0 a	2.7 b	77.8 a	12.1 a	4.9 a	17.0 a	8.2 c	4.5 a	12.7 b
A11B2	9.7 b	3.9 a	62.0 a	11.2 a	4.9 a	16.2 a	12.3 a	5.0 a	17.6 a
A11B5	11.6 a	4.2 a	64.2 a	10.3 a	4.9 a	15.4 a	9.2 c	4.3 a	13.6 b
A13B1	11.3 a	4.1 a	71.3 a	11.9 a	5.1 a	17.0 a	9.0 c	4.2 a	13.0 b
A13B2	13.4 a	2.5 b	63.1 a	11.0 a	5.0 a	16.0 a	11.1 b	4.6 a	16.0 a
A13B3	6.61 b	1.3 c	77.3 a	10.9 a	4.9 a	15.90 a	8.4 c	4.8 a	13.3 b
CV (%)	47.2	41.2	23.3	17.4	14.6	11.1	16.3	23.1	16.0

Means followed by the same letter in the column do not differ statistically by the Scott-Knott Test ($p > 0.05$). SG: seed germination, RDL: radicle length, RL: root length, SL: shoot length, TL: total length, SDW: shoot dry mass, RDW: root dry mass, TDW: total dry mass.

Table 4 - Effect of *Bacillus* spp. strains on sourgrass (*Digitaria insularis*) control in laboratory and greenhouse conditions.

Strains	Laboratory experiment		Greenhouse experiment						
	SG (%)	RDL (cm)	SG (%)	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
Control	40.0 a	5.2 a	26.6 a	11.1 a	1.2 a	12.3 a	2.0 a	2.5 a	4.5 a
A1B1	26.6 a	1.6 c	25.6 a	11.2 a	1.2 a	12.4 a	1.9 a	2.3 a	4.2 a
A1B3	22.6 b	2.5 b	27.1 a	12.2 a	1.3 a	13.6 a	2.3 a	2.0 a	4.2 a
A1B4	24.0 b	3.1 a	34.5 a	12.4 a	1.2 a	13.6 a	1.8 a	2.1 a	4.0 a
A1B5	33.3 a	3.5 a	26.1 a	10.3 a	1.3 a	11.8 a	2.2 a	3.0 a	5.3 a
A2B1	26.6 a	1.5 c	31.2 a	9.9 a	1.4 a	11.4 a	2.5 a	2.2 a	4.6 a
A2B2	17.3 b	3.9 a	27.7 a	11.3 a	1.1 a	12.4 a	1.9 a	2.7 a	4.6 a
A2B3	16.0 b	4.1 a	31.6 a	10.5 a	1.3 a	11.8 a	2.0 a	2.4 a	4.5 a
A5B1	16.0 b	2.5 b	27.8 a	10.2 a	1.1 a	11.4 a	1.8 a	2.4 a	4.3 a
A5B3	21.3 b	3.8 a	28.9 a	10.1 a	1.3 a	11.5 a	2.1 a	2.7 a	4.8 a
A5B4	20.0 b	2.9 b	22.8 a	9.9 a	1.2 a	11.2 a	2.1 a	2.6 a	4.7 a
A10B1	20.0 b	2.7 b	33.6 a	10.7 a	1.5 a	12.2 a	2.2 a	3.1 a	5.4 a
A10B4	30.6 a	3.6 a	32.8 a	11.1 a	1.6 a	12.7 a	2.8 a	2.5 a	5.3 a
A10B5	36.0 a	1.1 c	25.4 a	11.6 a	1.3 a	13.0 a	2.1 a	2.5 a	4.6 a
A11B1	13.3 b	1.3 c	24.0 a	9.3 a	1.2 a	10.5 a	2.1 a	2.6 a	4.8 a
A11B2	17.3 b	4.2 a	18.6 a	10.8 a	1.3 a	12.2 a	1.5 a	1.9 a	3.4 a
A11B5	21.3 b	2.5 b	17.0 a	9.4 a	0.8 a	10.3 a	1.2 a	1.1 a	2.2 a
A13B1	21.3 b	2.4 b	19.6 a	10.6 a	1.1 a	11.7 a	2.4 a	3.8 a	6.2 a
A13B2	28.0 a	1.5 c	37.7 a	11.5 a	1.2 a	12.7 a	1.5 a	1.3 a	2.8 a
A13B3	34.6 a	2.6 b	31.6 a	9.5 a	1.4 a	11.0 a	1.8 a	2.3 a	4.2 a
CV(%)	33.8	41.2	37.7	16.3	19.4	15.2	33.4	35.7	30.1

Means followed by the same letter in the column do not differ statistically by the Scott-Knott Test ($p > 0.05$). SG:seed germination, RDL: radicle length, RL: root length, SL: shoot length, TL: total length, SDW: shoot dry mass, RDW: root dry mass, TDW: total dry mass

Seeds of *D. insularis* were sensitive to 12 *Bacillus* spp. isolates for germination and 12 *Bacillus* spp. isolates for *in vitro* root growth (Table 4). Germination decreased, on average, by 52%, while radicle length had an average decrease of 59.3%. Despite promising laboratory results, none of the isolates were able to cause deleterious effects on germination or seedling growth under greenhouse conditions (Table 4). This fact may be related to the different fermentation methodologies of *Bacillus* spp. isolates for both assays. In the laboratory, when using static fermentation, *Bacillus* spp. isolates reached concentrations between 10^4 and 10^5 CFU mL^{-1}, values much lower than those obtained in greenhouse experiments (10^8 and 10^9 CFU mL^{-1}). Considering that the rate of isolates was 1 mL per Petri dish (concentrations between 10^4 and 10^5 CFU mL^{-1}) in laboratory assays and 1.0 x 10^7 CFU $container^{-1}$ in greenhouse assays, it is possible to infer that the concentration of bacterial cells may not be the most important factor in the inhibitory effect of these *Bacillus* spp. isolates on the germination or growth of *D. insularis* plants. Bioassays can evaluate the production of plant-suppressive compounds by rhizobacteria. An example of these compounds is the hydrocyanic acid, a toxic gas for plant metabolism (Kennedy et al., 2001). Thus, the different methods for obtaining isolates may have altered the production of these compounds, and the direct *in vitro* contact of the seed with the bacterium may have optimized the suppression effect.

The sensitivity of *C. sumatrensis* to the antagonistic action of *Bacillus* spp. was very high under laboratory conditions (Table 5). A total of 14 isolates reduced germination of *C. sumatrensis* seeds, with an average inhibition of 37.2%. In addition, all isolates decreased root length (Table 5). Isolate A10B4 was the most effective in reducing seed germination under laboratory conditions (60.3% inhibition), being also one of the isolates that most reduced radicle length (73.3% inhibition).

Table 5 - Effect of *Bacillus* spp. strains on horseweed (*Conyza sumatrensis*) control in laboratory and greenhouse conditions.

Strains	Laboratory experiment		Greenhouse experiment						
	SG (%)	RDL (cm)	SG (%)	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
Control	70.6 a	1.3 a	33.4 a	7.9 b	0.8 a	8.8 b	0.6 a	0.4 a	1.1 a
A1B1	51.6 b	0.3 d	22.6 b	9.3 a	0.8 a	10.1 a	0.6 a	0.4 a	1.1 a
A1B3	64.0 a	0.5 c	27.6 b	9.2 a	0.9 a	10.2 a	1.9 a	0.4 a	2.1 a
A1B4	54.6 b	0.5 c	18.9 b	8.3 a	0.7 a	9.0 b	0.8 a	0.4 a	1.2 a
A1B5	52.0 b	1.0 b	40.6 a	7.9 b	0.6 a	8.6 b	0.6 a	0.7 a	1.3 a
A2B1	65.3 a	0.6 c	43.1 a	7.5 b	0.8 a	8.4 b	0.5 a	0.4 a	0.9 a
A2B2	46.6 b	0.4 d	15.4 b	6.4 b	0.9 a	7.3 b	0.8 a	0.4 a	1.3 a
A2B3	53.3 b	0.2 d	20.2 b	7.4 b	0.7 a	8.2 b	0.8 a	0.4 a	1.2 a
A5B1	53.3 b	0.5 c	33.8 a	7.7 b	0.7 a	8.5 b	0.7 a	0.5 a	1.2 a
A5B3	54.6 b	0.5 c	29.9 a	9.2 a	0.7 a	10.0 a	0.7 a	0.5 a	1.3 a
A5B4	50.6 b	0.3 d	33.1 a	7.2 b	0.8 a	8.0 b	0.5 a	0.5 a	1.0 a
A10B1	50.6 b	0.5 c	19.8 b	7.2 b	0.7 a	8.0 b	0.7 a	0.4 a	1.1 a
A10B4	28.0 c	0.3 d	39.6 a	8.0 b	0.9 a	9.0 b	0.7 a	0.5 a	1.3 a
A10B5	69.3 a	0.4 c	16.1 b	8.9 a	0.7 a	9.7 a	0.7 a	0.4 a	1.1 a
A11B1	62.6 a	0.4 c	25.3 b	9.3 a	0.8 a	10.1 a	0.7 a	0.5 a	1.3 a
A11B2	64.0 a	0.4 c	56.5 a	8.0 b	0.8 a	8.9 b	0.6 a	0.4 a	1.1 a
A11B5	50.6 b	0.3 d	51.4 a	8.7 a	0.8 a	9.5 a	0.5 a	0.3 a	0.9 a
A13B1	37.3 c	0.3 d	33.1 a	8.3 a	0.9 a	9.2 a	0.7 a	0.7 a	1.4 a
A13B2	44.0 b	0.4 c	38.3 a	8.9 a	0.9 a	9.8 a	0.7 a	0.4 a	1.2 a
A13B3	46.6 b	0.4 c	21.4 b	7.5 b	0.9 a	8.4 b	2.4 a	0.4 a	2.9 a
CV (%)	24.1	30.9	44.1	14.6	20.7	13.3	128.1	51.9	78.6

Means followed by the same letter in the column do not differ statistically by the Scott-Knott Test ($p > 0.05$). SG:seed germination, RDL: radicle length, RL: root length, SL: shoot length, TL: total length, SDW: shoot dry mass, RDW: root dry mass, TDW: total dry mass

In the greenhouse experiment, nine *Bacillus* spp. isolates decreased seed germination, with an average inhibition of 37.7%, but none was able to reduce root growth (Table 5). On the other hand, isolates A1B1, A1B3, A1B4, A10B5, and A11B1 promoted root growth, showing that the mechanism of action in inhibiting germination for these isolates may not be related to bacterial IAA secretion, since the concentration that reduces germination is also detrimental to root growth (Chauhan et al., 2009). A similar result was found by Carvalho et al. (2011) with isolate 80-20 from *Bacillus pumilus* Meyer and Gottheil, 1901. This isolate was one of the most efficient in inhibiting lettuce seed germination; however, it was the only one among 25 isolates that did not reduce the growth of wheat coleoptiles.

Isolates A1B3, A10B5, and A11B1 from *Bacillus* spp. did not present an *in vitro* inhibitory effect on the germination of *C. sumatrensis* seeds, but reduced seed germination under greenhouse conditions. Three factors can be considered as the possible causes of this effect. The first is the temperature difference between the two experimental conditions; the second is the different methods of producing bacterial isolates in the respective experiments; and the third is the rate applied per experimental unit (Petri dish or container). The temperature conditions in the greenhouse may have benefited the development of bacterial strains, since it varied between 18 and 29 °C, reaching values close to the fermentation temperature (29 °C). Laboratory conditions, in turn, included the constant temperature of 22 °C, considered favorable to the germination of horseweed seeds (Nandula et al., 2006). Regarding the production of strains, the different methodologies may have altered the secretion of metabolites such as hydrocyanic acid by bacteria, as previously mentioned. Furthermore, the rate of isolates was different for both assays. In the laboratory assay, the rate was 10^4-10^5 CFU Petri plate^{-1}; for greenhouse assays, the rate was 1.0×10^7 CFU container^{-1}. According to Begonia et al. (1998), bacterial cell concentration plays a key role in the deleterious effect on

weeds, as it can directly influence root colonization by bacteria. These authors verified germination inhibition and gradual reduction in seedling growth with the application of supernatant-free fluorescent bacteria isolates at rates of 10^7, 10^8, and 10^9 CFU g soil^{-1}. Isolate VLBR-01 reduced seed germination and root growth in the three rates tested, while isolate V239 reduced germination only at the rate of 10^9 CFU g soil^{-1}. In this same study, bacterial concentrations in the root, rhizosphere, and soil followed the same trend of the applied rates.

The main difference in cultivation between the fermentation methods of the bacterial isolates is the oxygenation of the culture medium, provided by the shaking process in the method defined for greenhouse experiments. Oxygen is the essential element in most metabolic processes of aerobic organisms because it is an electron acceptor. Its deficiency can lead to reduced efficiency of NADH and NADPH oxidation, essential reactions for ATP production, and low flow through the tricarboxylic acid (TCA) cycle (Zhang & Xiu, 2009). Katzer et al. (2001) found that the production of secondary metabolites such as hydrocyanic acid by bacteria is not only influenced by factors such as restriction of nitrogen or phosphate, but also may depend on oxygen limitation during the fermentation process.

Six isolates of *Pseudomonas* spp. significantly reduced the *in vitro* germination of *E. heterophylla* seeds, with an average inhibition of 80.3%. These isolates were: A1P6, A4XP4, A8P2, A8P8, A8XP10, and A9P2 (Table 6). With the exception of isolate A9P2, these isolates also reduced seedling root length, as observed for these isolates (A1XP10, A3XP14, A8P4, A10P5, A18XP4, A14P4, A14P2, A13XP8, A11P12, A11P11) (Table 6).

Table 6 - Effect of *Pseudomonas* spp. strains on wild poinsettia (*Euphorbia heterophylla*) control in laboratory and greenhouse conditions.

Strains	Laboratory. experiment		Greenhouse experiment						
	SG (%)	RDL (cm)	SG (%)	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
Control	56.0 a	7.5 a	52.0 a	14.8 a	3.5 a	18.3 a	18.5 a	8.9 b	27.4 a
A1P6	12.2 b	2.1 b	45.5 a	16.2 a	3.5 a	19.6 a	19.9 a	8.6 b	28.4 a
A1XP10	52.0 a	2.2 b	45.3 a	15.4 a	3.5 a	18.9 a	14.1 b	8.6 b	22.8 a
A2P6	38.8 a	4.6 a	57.0 a	14.0 a	3.4 a	17.4 a	18.2 a	8.6 b	26.7 a
A2XP3	49.3 a	4.1 a	52 .0 a	15.1 a	3.4 a	18.5 a	17.8 a	8.7 b	26.6 a
A3P2	52.5 a	5.5 a	58.0 a	15.8 a	3.6 a	19.3 a	18.1 a	7.6 b	25.4 a
A3P6	52.5 a	4.9 a	51.0 a	15.6 a	3.3 a	18.9 a	17.8 a	7.9 b	25.5 a
A3XP10	45.7 a	6.1 a	52.5 a	13.4 a	3.4 a	16.9 a	16.6 b	6.3 b	22.3 a
A3XP11	44.5 a	7.1 a	53.0 a	14.2 a	3.3 a	17.4 a	15.3 b	7.8 b	23.2 a
A3XP12	45.7 a	5.6 a	39.0 a	11.5 a	3.6 a	15.4 a	17.0 b	7.8 b	24.7 a
A3XP14	40.0 a	1.3 b	50.7 a	15.9 a	3.7 a	19.7 a	13.7 b	8.3 b	22.0 a
A3XP15	40.0 a	5.1 a	47.0 a	14.3 a	3.5 a	17.8 a	18.2 a	10.3 a	29.1 a
A4XP4	18.6 b	1.1 b	49.3 a	14.9 a	3.5 a	18.4 a	18.3 a	8.6 b	26.8 a
A7P1	42.2 a	4.6 a	55.5 a	16.7 a	3.6 a	20.2 a	16.5 b	10.8 a	28.5 a
A8P2	3.5 b	1.9 b	48.4 a	12.9 a	3.7 a	16.8 a	19.6 a	7.1 b	26.5 a
A8P4	46.6 a	1.7 b	40.0 a	11.3 a	2.9 a	14.1 a	15.5 b	6.5 b	21.5 a
A8P8	15.7 b	1.2 b	49.8 a	14.0 a	3.2 a	17.1 a	19.9 a	7.1 b	26.9 a
A8XP10	5.3 b	0.5 b	50.7 a	14.5 a	3.5 a	18.0 a	14.9 b	8.9 b	23.9 a
A8XP9	35.4 a	6.8 a	54.3 a	17.3 a	3.6 a	20.7 a	16.8 b	9.4 b	26.7 a
A9P1	36.5 a	4.4 a	63.6 a	14.3 a	3.5 a	17.9 a	17.9 a	7.6 b	25.1 a
A9P2	10.5 b	4.2 a	42.6 a	15.0 a	4.0 a	19.1 a	20.6 a	8.9 b	29.4 a
A9XP2	44.5 a	4.9 a	47.4 a	15.6 a	3.5 a	19.0 a	18.5 a	11.5 a	31.0 a
A10P5	30.6 a	3.7 b	42.7 a	13.6 a	3.7 a	17.2 a	19.6 a	7.9 b	26.6 a
A10XP5	44.5 a	5.5 a	62.4 a	15.5 a	3.6 a	19.1 a	15.7 b	7.4 b	23.1 a
A11P11	42.6 a	2.8 b	46.7 a	15.0 a	3.5 a	18.5 a	18.4 a	8.6 b	26.9 a
A11P12	53.3 a	2.4 b	40.0 a	13.4 a	3.6 a	16.9 a	19.2 a	7.7 b	26.1 a

Cont. Table 6

Strains	Laboratory. experiment		Greenhouse experiment						
	SG (%)	RDL (cm)	SG (%)	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
A11XP1	44.0 a	4.6 a	36.0 a	13.1 a	3.6 a	16.6 a	19.1 a	7.6 b	25.8 a
A11XP13	28.5 a	5.5 a	52.0 a	15.9 a	3.8 a	19.7 a	22.1 a	12.2 a	35.0 a
A13XP8	32.0 a	0.8 b	57.3 a	15.7 a	3.9 a	19.6 a	17.8 a	9.6 b	26.6 a
A14P2	34.6 a	1.1 b	54.7 a	15.4 a	3.9 a	19.3 a	15.8 b	8.4 b	24.3 a
A14P4	46.6 a	1.6 b	61.3 a	14.3 a	3.8 a	18.2 a	14.5 b	8.3 b	22.9 a
A16P4	33.2 a	5.0 a	39.0 a	14.8 a	3.8 a	18.7 a	20.3 a	8.6 b	28.8 a
A18XP4	42.6 a	1.8 b	56.7 a	16.3 a	3.8 a	20.1 a	15.0 b	9.1 b	24.0 a
A19XP2	45.6 a	4.6 a	52.0 a	18.2 a	3.6 a	21.6 a	18.5 a	14.4 a	35.3 a
CV (%)	49.1	49.3	25.6	19.3	15.8	16.6	19.2	30.1	21.8

Means followed by the same letter in the column do not differ statistically by the Scott-Knott Test ($p > 0.05$). SG: seed germination, RDL: radicle length, RL: root length, SL: shoot length, TL: total length, SDW: shoot dry mass, RDW: root dry mass, TDW: total dry mass

Under greenhouse conditions, isolates reduced neither seed germination nor root and shoot length of *E. heterophylla* plants (Table 6). However, 13 isolates reduced SDW, which showed an average inhibition of 16.2%. Five isolates promoted increased RDW (A3XP15, A7P1, A9XP2, A11XP13, and A19XP2). Some *Bacillus* spp. showed similar behavior, except that none of these reduced germination or growth. Despite having a growth promoting effect on *E. heterophylla*, these isolates inhibited the germination of *C. sumatrensis* (Table 8), and some of them inhibited germination in *D. insularis* (Table 7). Different types of plants may influence the behavior of DRB, either directly with colonization, growth, and physiology, or indirectly by affecting native microflora (Åström & Gerhardson, 1988). Isolate D7 from *Pseudomonas fluorescens* (Migula, 1895), for example, was shown to inhibit the germination of several weed species; notwithstanding, it promoted growth of the weed species *Brassica napus* L. (Kennedy et al., 2001).

Table 7 - Effect of *Pseudomonas* spp. strains on sourgrass (*Digitaria insularis*) control in laboratory and greenhouse conditions.

Strains	Laboratory experiment		Greenhouse experiment						
	SG %	RDL (cm)	SG %	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
Control	42.6 a	0.9 a	42.5 a	9.8 a	0.8 a	10.6 a	0.98 b	1.3 b	2.3 b
A1P6	38.7 a	0.7 a	34.5 a	10.3 a	0.7 a	11.0 a	1.39 a	1.5 a	2.9 a
A1XP10	24.0 b	0.3 b	53.9 a	9.3 b	0.8 a	10.2 b	0.96 b	1.2 b	2.1 b
A2P6	29.2 b	0.9 a	44.6 a	9.1 b	0.8 a	10.0 b	0.94 b	0.9 b	1.8 b
A2XP3	57.3 a	1.0 a	41.0 a	8.6 b	0.7 a	9.4 b	1.01 b	1.0 b	2.0 b
A3P2	19.3 b	0.7 a	45.4 a	8.7 b	0.6 a	9.4 b	0.90 b	0.9 b	1.7 b
A3P6	35.3 a	0.9 a	38.4 a	8.5 b	0.6 a	9.2 b	0.80 b	0.9 b	1.7 b
A3XP10	41.4 a	0.8 a	37.1 a	9.2 b	0.6 a	9.8 b	0.78 b	0.9 b	1.6 b
A3XP11	31.7 b	0.5 b	48.5 a	8.9 b	0.9 a	9.9 b	0.98 b	0.9 b	1.8 b
A3XP12	4.8 b	0.2 b	39.7 a	10.1 a	0.8 a	11.0 a	0.90 b	0.9 b	1.8 b
A3XP14	4.8 b	0.2 b	34.9 a	8.6 b	0.7 a	9.3 b	0.89 b	0.8 b	1.7 b
A3XP15	42.6 a	0.8 a	35.0 a	8.6 b	0.8 a	9.5 b	1.00 b	1.1 b	2.0 b
A4XP4	41.3 a	0.9 a	49.1 a	8.4 b	0.7 a	9.2 b	1.00 b	1.0 b	2.0 b
A7P1	21.9 b	0.5 b	42.9 a	9.3 b	0.8 a	10.2 b	1.08 b	1.2 b	2.3 b
A8P2	27.1 b	0.3 b	35.5 a	10.3 a	0.7 a	11.0 a	1.31 a	1.6 a	2.9 a
A8P4	21.3 b	0.6 b	39.7 a	8.9 b	0.8 a	9.7 b	0.91 b	0.9 b	1.8 b
A8P8	50.6 a	0.5 b	41.3 a	11.7 a	0.7 a	12.4 a	1.25 a	1.5 a	2.8 a

Cont. Table 7

Strains	Laboratory experiment		Greenhouse experiment						
	SG	RDL	SG	RL	SL	TL	SDW	RDW	TDW
	%	(cm)	%	(cm)	(cm)	(cm)	(g)	(g)	(g)
A8XP10	29.1 b	0.4 b	49.6 a	7.8 b	0.7 a	8.6 b	1.02 b	1.0 b	2.0 b
A8XP9	43.8 a	0.9 a	33.6 a	9.6 a	1.0 a	10.8 a	1.24 a	1.6 a	2.9 a
A9P1	40.2 a	0.9 a	39.1 a	9.6 a	1.0 a	10.6 a	1.14 a	1.2 b	2.3 b
A9P2	38.7 a	0.4 b	39.8 a	10.1 a	0.7 a	10.8 a	1.34 a	1.8 a	3.1 a
A9XP2	15.8 b	0.4 b	38.4 a	10.8 a	0.8 a	11.7 a	1.08 b	1.3 b	2.4 b
A10P5	46.6 a	0.7 a	38.9 a	9.4 b	0.7 a	10.1 b	0.97 b	1.2 b	2.2 b
A10XP5	46.3 a	0.8 a	33.6 a	8.9 b	0.9 a	9.9 b	1.18 a	1.4 b	2.6 a
A11P11	50.6 a	0.5 b	43.5 a	9.0 b	0.8 a	9.9 b	0.88 b	1.0 b	1.9 b
A11P12	41.3 a	0.7 a	44.8 a	9.1 b	0.8 a	10.0 b	0.91 b	1.1 b	2.0 b
A11XP1	48.0 a	0.6 a	38.2 a	8.6 b	0.8 a	9.4 b	1.01 b	1.0 b	2.1 b
A11XP13	35.3 a	0.7 a	37.7 a	10.1 a	0.8 a	11.0 a	1.03 b	1.2 b	2.2 b
A13XP8	38.7 a	0.4 b	45.0 a	8.5 b	0.7 a	9.3 b	1.00 b	1.0 b	2.0 b
A14P2	40.7 a	0.5 b	49.1 a	8.7 b	0.7 a	9.5 b	0.98 b	0.8 b	1.8 b
A14P4	32.9 a	0.3 b	43.8 a	8.1 b	0.7 a	8.8 b	0.98 b	1.0 b	2.0 b
A16P4	25.2 b	0.5 b	38.5 a	10.3 a	0.7 a	11.0 a	1.41 a	1.8 a	3.2 a
A18XP4	34.9 a	0.5 b	47.3 a	9.2 b	0.8 a	10.0 b	0.85 b	0.9 b	1.8 b
A19XP2	20.7 b	0.3 b	39.3 a	9.6 a	0.7 a	10.3 a	1.01 b	1.2 b	2.2 b
CV(%)	50.7	45.5	26.8	17.3	22.7	16.0	28.5	51.4	31.3

Means followed by the same letter in the column do not differ statistically by the Scott-Knott Test ($p > 0.05$). SG: seed germination, RDL: radicle length, RL: root length, SL: shoot length, TL: total length, SDW: shoot dry mass, RDW: root dry mass, TDW: total dry mass

Seeds of *D. insularis* had an average *in vitro* germination reduction of 50.35%, caused by 13 isolates of *Pseudomonas* spp. (Table 7). Most of these isolates also decreased radicle growth in *D. insularis* seedlings, except for isolates A2P6 and A3P2. Moreover, seven other isolates also decreased radicle growth (Table 7). The germination of *D. insularis* seeds was not decreased by application of *Pseudomonas* spp. isolates in greenhouse assays (Table 7); however, 22 of the tested isolates decreased root length. Similarly to the previous assays of *E. heterophylla* with application of *Pseudomonas* spp., isolates that had no effect on the reduction of seedling growth under laboratory conditions reduced the root length of plants in the greenhouse (Table 7). Eight isolates showed plant growth-promoting effect (DSW and RDW). Of these, isolates A8P2 and A16P4 reduced germination and seedling length in the laboratory. Isolates A8P8 and A9P1, in turn, reduced only root length in the laboratory assay, showing that the response to these isolates also appears to be related to the concentration of IAA by having a rate-dependent effect to inhibit germination or promote seedling growth.

Seeds of *C. sumatrensis* were very susceptible to *Pseudomonas* spp. in the laboratory assay (Table 8). Germination decreased in 75% of the tested isolates (25 isolates), with an average inhibition of 50.3%. Most of these isolates also decreased seedling radicle length (Table 8). On the contrary, in greenhouse assays, germination increased with the application of 18 isolates (54%). Fifteen of these isolates inhibited germination or decreased seedling growth in the laboratory (Table 8). Increased wheat germination by DRB isolates was reported by Abbas et al. (2017), in which isolates T19, L9, and $7O_0$ significantly increased germination by 14.8, 18.5, and 14.8% compared to the control, respectively.

Table 8 - Effect of *Pseudomonas* spp. strains on horseweed (*Conyza sumatrensis*) control in laboratory and greenhouse conditions.

Strains	Laboratory experiment		Greenhouse experiment						
	SG	RDL	GS	RL	SL	TL	SDW	RDW	TDW
	(%)	(cm)	(%)	(cm)	(cm)	(cm)	(g)	(g)	(g)
Control	32.0 a	0.4 a	3.7 b	5.5 a	0.2 a	5.7 a	0.34 a	0.3 a	0.6 a
A1P6	28.2 b	0.3 a	2.1 b	5.8 a	0.2 a	6.0 a	0.40 a	0.5 a	0.9 a
A1XP10	34.6 a	0.2 a	5.5 a	7.4 a	0.2 a	7.6 a	0.35 a	0.2 a	0.5 a

Cont. Table 8

Strains	Laboratory experiment		Greenhouse experiment						
	SG (%)	RDL (cm)	GS (%)	RL (cm)	SL (cm)	TL (cm)	SDW (g)	RDW (g)	TDW (g)
A2P6	8.7 b	0.2 b	3.4 b	5.3 a	0.2 a	5.6 a	0.4 a	0.3 a	0.7 a
A2XP3	12.0 b	0.1 b	5.2 a	6.5 a	0.2 a	6.7 a	0.4 a	0.3 a	0.7 a
A3P2	13.5 b	0.1 b	2.8 b	5.5 a	0.2 a	5.7 a	0.3 a	0.2 a	0.5 a
A3P6	8.7 b	0.1 b	3.2 b	5.2 a	0.3 a	5.5 a	0.3 a	0.2 a	0.6 a
A3XP10	4.8 b	0.1 b	3.2 b	5.8 a	0.2 a	6.1 a	0.3 a	0.2 a	0.5 a
A3XP11	13.5 b	0.2 a	3.2 b	5.4 a	0.2 a	5.6 a	0.3 a	0.2 a	0.6 a
A3XP12	1.9 b	0.2 b	2.7 b	5.8 a	0.2 a	6.0 a	0.3 a	0.2 a	0.6 a
A3XP14	22.6 b	0.2 b	2.7 b	5.6 a	0.2 a	6.0 a	0.2 a	0.3 a	0.5 a
A3XP15	9.7 b	0.1 b	3.8 a	5.2 a	0.2 a	5.4 a	0.4 a	0.2 a	0.6 a
A4XP4	26.6 b	0.1 b	4.4 a	4.6 a	0.2 a	4.8 a	0.2 a	0.2 a	0.4 a
A7P1	15.5 b	0.1 b	4.2 a	5.9 a	0.2 a	6.1 a	0.4 a	0.2 a	0.6 a
A8P2	22.5 b	0.2 a	2.7 b	5.9 a	0.2 a	6.2 a	0.4 a	0.4 a	0.9 a
A8P4	13.3 b	0.7 b	5.1 a	6.8 a	0.2 a	7.0 a	0.3 a	0.3 a	0.7 a
A8P8	20.7 b	0.25 a	2.4 b	4.8 a	0.2 a	5.0 a	0.4 a	0.2 a	0.6 a
A8XP10	20.0 b	0.09 b	5.9 a	6.7 a	0.2 a	6.9 a	0.4 a	0.2 a	0.6 a
A8XP9	8.7 b	0.18 b	4.2 a	4.8 a	0.2 a	5.1 a	0.4 a	0.2 a	0.6 a
A9P1	19.3 b	0.15 b	5.2 a	5.0 a	0.2 a	5.3 a	0.4 a	0.2 a	0.5 a
A9P2	50.8 a	0.28 a	3.0 b	4.6 a	0.2 a	4.9 a	0.3 a	0.2 a	0.6 a
A9XP2	20.3 b	0.24 a	4.0 a	5.3 a	0.2 a	5.5 a	0.4 a	0.2 a	0.6 a
A10P5	34.6 a	0.22 a	2.9 b	5.7 a	0.2 a	5.9 a	0.7 a	0.6 a	1.3 a
A10XP5	11.6 b	0.24 a	4.4 a	5.4 a	0.3 a	5.7 a	0.4 a	0.2 a	0.6 a
A11P11	40.0 a	0.12 b	2.6 b	6.4 a	0.2 a	6.6 a	0.3 a	0.5 a	0.8 a
A11P12	22.6 b	0.21 b	4.4 a	6.9 a	0.2 a	7.2 a	0.4 a	0.3 a	0.7 a
A11XP1	37.3 a	0.30 a	3.0 b	6.0 a	0.2 a	6.2 a	0.3 a	0.4 a	0.7 a
A11XP13	18.4 b	0.28 a	4.0 a	6.7 a	0.2 a	7.0 a	0.3 a	0.2 a	0.5 a
A13XP8	16.0 b	0.15 b	6.3 a	7.0 a	0.2 a	7.3 a	0.3 a	0.2 a	0.5 a
A14P2	36.0 a	0.17 b	4.3 a	7.0 a	0.2 a	7.2 a	0.3 a	0.3 a	0.6 a
A14P4	14.6 b	0.09 b	2.0 b	6.0 a	0.2 a	6.3 a	0.3 a	0.4 a	0.9 a
A16P4	33.8 a	0.30 a	5.0 a	8.0 a	0.2 a	8.3 a	0.4 a	0.3 a	0.7 a
A18XP4	24.0 b	0.13 b	4.6 a	5.4 a	0.2 a	5.6 a	0.3 a	0.3 a	0.6 a
A19XP2	63.5 a	0.38 a	4.3 a	5.3 a	0.3 a	5.6 a	0.4 a	0.2 a	0.5 a
CV (%)	102.2	67.3	44.2	26.4	17.0	25.1	44.4	61.5	47.7

Means followed by the same letter in the column do not differ statistically by the Scott-Knott Test ($p > 0.05$). SG:seed germination, RDL: radicle length, RL: root length, SL: shoot length, TL: total length, SDW: shoot dry mass, RDW: root dry mass, TDW: total dry mass

For the three weed species studied, radicle growth seems to be more affected by bacterial application than germination under both *in vitro* and greenhouse conditions. The same can be observed in the study of Kennedy et al. (2001) with *P. fluorescens* D7 supernatant on various weed species. Of the 26 plant species evaluated in Petri dishes with agar-water, 19 inhibited root growth, while only 10 showed inhibition of germination.

We can infer from this study that the mass selection of rhizobacteria for pre-emergence control of weeds by the *in vitro* methodology tested may not be the most adequate for the selection of isolates. Most of the time, the efficiency of some *in vitro* isolates was not corroborated by greenhouse experiments. Similar results were found by Kennedy et al. (2001) using isolate *Pseudomonas fluorescens* D7. In their study, no correlation was found between the bioassay with plate supernatants and the responses found in the soil bioassay ($R^2 = 0.18$), since root inhibition was always higher in agar-water than in soil. The authors attributed this effect to the ability of competition and colonization of the rhizosphere by bacteria in soil compared to agar-water medium.

Inhibition of seed germination is the main and most sought-after effect of DRBs for biological control of weeds due to the ability to reduce plant population in the field (seed bank). Considering the inhibition of seed germination, we conclude that *Bacillus* spp. isolates A1B1, A1B3, A1B4, A2B2, A2B3, A10B1, A10B5, A11B1, and A13B3 were able to effectively inhibit the germination of *C. sumatrensis* seeds under greenhouse conditions. These isolates are promising and should be identified at the species level and characterized for metabolite production, plant growth regulation, root colonization, and effect on cultivated plants.

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