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Influence of plant growth-promoting rhizobacteria (PGPR) on blackberry (*Rubus glaucus* Benth. cv. thornless) growth under semi-cover and field conditions

Influencia de rizobacterias promotoras del crecimiento vegetal (PGPR) en el crecimiento de la mora (*Rubus glaucus* Benth. cv. sin espinas) bajo semitecho y libre exposición

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Abstract

This study shows the effect of three plant growth-promoting rhizobacteria (PGPR) strains of the genus *Bacillus* on blackberry (*Rubus glaucus* Benth.) development under semi cover and field conditions (cropping systems). Endogenous strain *Bacillus subtilis* GIBI-200, *B. pumilus* GIBI-206, its mixture (*B. subtilis* GIBI-200 + *B. pumilus* GIBI-206), and commercial strain *B. subtilis* QST-713 were compared to conventional mineral fertilization (Control) in each cropping system. Field condition system presented higher values ($P<0.001$) than semi cover in: total number of branches (7.32), number of productive branches (7.0), flowers per raceme (26.2), and the lowest percentage of unproductive branches (6.1%). Significant differences ($P<0.05$) were obtained in total number of branches over time by bacterial strains. Nonetheless, last observation of this variable did not present statistical differences among strains and mineral fertilization. No significant differences were evident in terms of number, length, diameter and flowers in the productive branches regarding to bacterial strains and Control. *B. subtilis* GIBI-200 + *B. pumilus* GIBI-206 showed a similar statistical behavior compare to mineral fertilization in the number of clusters (8.3) and percentage of unproductive branches (11.1%). In addition, GIBI-200 + GIBI-206, despite statistical equality, exposed greater values than individual strains. Field condition system remains the most promising alternative for the development of blackberry crop and PGPR acts as biofertilizers achieving effects in the long-term growth of blackberry similar to mineral fertilization. Mixtures of PGPR can produce a synergic effect and new combinations should be evaluated in future studies.

Key words: biofertilizers; cropping system; fruitculture; plastic film covers; soil microorganisms; symbiosis.

Resumen

Este estudio muestra el efecto de tres cepas de rizobacterias promotoras del crecimiento vegetal (PGPR) del género *Bacillus* sobre el desarrollo de la mora (*Rubus glaucus* Benth) bajo condiciones de semitecho y libre exposición (sistemas de cultivo). La cepa endógena *Bacillus subtilis* GIBI-200, *B. pumilus* GIBI-206, su mezcla (*B. subtilis* GIBI-200 + *B. pumilus* GIBI-206) y la cepa comercial *B. subtilis* QST-713 se compararon con la fertilización mineral convencional (Control) en cada sistema de cultivo. El sistema a libre exposición presentó valores más altos ($P <0.001$) que el semitecho en: número total de ramas (7.32), número de ramas productivas (7.0), flores por racimo (26.2) y menor porcentaje de ramas improductivas (6.1%). Se obtuvo diferencias significativas ($P <0.05$) en el número total de ramas a través del tiempo como efecto de las cepas bacterianas. No obstante, la última observación de esta variable no presentó diferencias estadísticas entre cepas y fertilización mineral. No se observaron diferencias significativas en términos de número, longitud, diámetro y flores en las ramas productivas como efecto de las cepas bacterianas y el Control. *B. subtilis* GIBI-200 + *B. pumilus* GIBI-206 mostró un comportamiento estadístico similar comparado con la fertilización mineral en el número de racimos (8.3) y porcentaje de ramas improductivas (11.1%). Además, GIBI-200 + GIBI-206, a pesar de la igualdad estadística, expuso mayores valores que las cepas individuales. El sistema a libre exposición sigue siendo la alternativa más prometedora para el desarrollo de cultivos de mora y PGPR actúan como biofertilizantes permitiendo lograr efectos en el crecimiento a largo plazo de mora, similar a la fertilización mineral. Las mezclas de PGPR pueden producir un efecto sinérgico y nuevas combinaciones deben ser evaluadas en futuros estudios.

Palabras clave: biofertilizantes; cubiertas de plástico; frutales; microorganismos del suelo; simbiosis; sistemas de cultivo.

Introduction

Blackberry (*Rubus glaucus* Benth.), also called Andean blackberry, is a perennial shrub belonging to the Rosaceae family native of The Andes of northern South America. This plant is commonly found in countries such as Ecuador, Colombia, Panama, Salvador, Honduras, Guatemala, Mexico, and the United States. In Colombia, the cultivation of this plant is an important source of income for small-scale rural producers. However, this productive activity has limitations in the disease-free propagating material, absence of breeding varieties with high productivity, indiscriminate use of agrochemicals, and poor research and development of technical packages making necessary the seeking of sustainable alternatives (Schreckinger, Lotton, Lila & Gonzales-de Mejía, 2010).

In agricultural production, the implementation of beneficial bacteria, generally associated with rhizosphere, is an initiative that promotes plant health and plant growth by acting as biofertilizers (Compant, Clément, & Sessitsch, 2010). Genera such as *Azospirillum, Azotobacter, Burkholderia, Enterobacter, Klebsiella, Pseudomonas* and *Bacillus* are some of the most prominent Plant Growth-Promoting Rhizobacteria (PGPR). Within the mechanisms of action of these bacteria are as follows: the synthesis of hormones such as indoleacetic acid (IAA) and reduction of ethylene, solubilization of phosphorus, nitrogen fixation, decreased toxicity of heavy metals, and biocontrol of plant diseases (Souza, Ambrosini & Passaglia, 2015).

Sustainability in agriculture involves maintaining high productivity through environmentally acceptable practices. Promissory alternatives for crop management through plastic films such as semi cover that controls, in a certain way, weather conditions are increasingly used. This technology helps to protect the plant from raindrop, have allowed a decreasing in the incidence of fungal diseases and improving crop productivity. In tropical regions, semi cover has shown positive results as a complement to technological packages of crops such as tomato (*Solanum lycopersicum* L.) (Gómez-Duque, Ceballos-Aguirre, Orozco-Castaño & Parra-Salinas, 2010).

Interest on crop responses are addressed to management strategies such as PGPR and cropping systems under semi cover. However, in species such as blackberry, research of these trends is scarce or nonexistent. Given these concerns, this study aimed to determine the effect of strains *Bacillus subtilis* GIBI-200, *B. pumilus* GIBI-206, *B. subtilis* GIBI-200 + *B. pumilus* GIBI-206, and *B. subtilis* QST-713 in plant growth and development of blackberry (*Rubus glaucus* Benth. cv. thornless) cultivation established in two cropping systems: under field conditions and semi cover in the department of Caldas, Colombia.

Materials and methods

Study area

The study was conducted in the Tesorito Farm of the Universidad de Caldas (Manizales-Caldas, Colombia) located at 2280 m.a.s.l, average temperature of 17ºC, relative humidity of 78%, average annual rainfall of 1800 mm, and solar radiation of 1215 h year^{-1}. The soils of the study area are of volcanic origin, mostly allophanic of the order Andisol with sandy loam texture.

Plant material

Blackberry cv. thornless (Figure 1 A), vegetatively propagated were used. This plant material was acquired in a commercial plantation in the municipality of Santa Rosa de Cabal-Risaralda, Colombia. *Rubus glaucus* Benth. cv. thornless reports productivities of 15 t.ha^{-1}.yr^{-1}, fruits with lengths of 3.5 cm, 2.3 cm diameter and average fruit weight ranged from 7.5 g to 8.5 g (Bernal & Diaz, 2006).

Semi cover system

It was built using bamboo stakes of 12 cm diameter buried in the ground to a soil depth of 65 cm. The distance between stakes was 3.5 m and 2 m between rows. The plastic cover (Agroclear®) had a thickness of 0.1524 mm, thermicity 55%, total light transmission 85% +/- 5, total light diffusion 55% +/- 5, UV block 290nm at 340nm (percentage of transmission) 2%, and UV light transmission at 340nm 1.5% (Figure 1 B).

Figure 1. Plant material and semi cover structure. **A.** Blackberry fruits (*Rubus glaucus* Benth cv. thornless) **B.** Semi cover structure for a blackberry crop.

PGPR- Plant growth-promoting rhizobacteria

The strains used were supplied by the microorganism collection of the Universidad Catolica de Manizales, corresponding to *Bacillus subtilis* GIBI-200 and *Bacillus pumilus* GIBI-206, respectively; isolated and characterized from lignocellulosic residues of *Ricinus communis* (Cabra, Meneses & Galeano, 2015). This strains were preserved in 10% glycerol at -80°C. Nutrient agar was employed to inoculate the strains and incubated for 48 h at 30ºC to confirm viability and purity. Subsequently, they were grown in nutrient broth, under constant stirring, at 150 rpm and 30ºC. Cell concentration was calculated by spectrophotometry at a wavelength (λ) of 600 nm to obtain a concentration of 10^8 cfu.ml^{-1}. *Bacillus subtilis* QST-713, a comercial strain, was also used. A backpack sprayer (RoyalCondor®), was used for plant inoculation, pouring 200 mL of inoculum at the base of each plant. This inoculation was performed three times at intervals of 5 months during the evaluation of this study.

Data analysis

A split plot design in completely randomized blocks was performed. Levels of main plot were the two cropping systems: semi cover and field conditions. The subplot consisted of bacterial strains as follows: *Bacillus subtilis* GIBI-200, *Bacillus pumillus* GIBI-206, mixture of GIBI-200 + GIBI-206, *Bacillus subtilis* QST-713 and Control or conventional treatment (1060 Kg of 15-15-15. ha^{-1}.yr^{-1}), divided into four blocks by main plot. The experimental unit was five plants. Data were analyzed using analysis of variance and average benchmarks type Duncan (P<0.05) through the GLM procedure of SAS® software. Total number of branches over time was measured by counting blackberry branches arising from the main plant stem. Percentage of unproductive branches, distance from main plant stem to apical meristem (Length) and thickness at the plant stem base (Diameter) of the productive branches were sampled at the start of first harvest, time from which there was a differentiation of these structures. The number of clusters was recorded by counting the clusters present in the basal, medial and apical sections of each branch. Finally, the number of flowers per cluster was calculated by averaging the number of flowers in random clusters in each branch section.

Results

Cropping Systems

Cropping systems did not present significant differences in the total number of branches from week 23 to week 41 after planting. At week 48, the last observation of this variable, significant differences (P<0.001) were obtained. Semi cover system acquired the lowest total number of branches (5.2) in week 48 after planting (Figure 2).

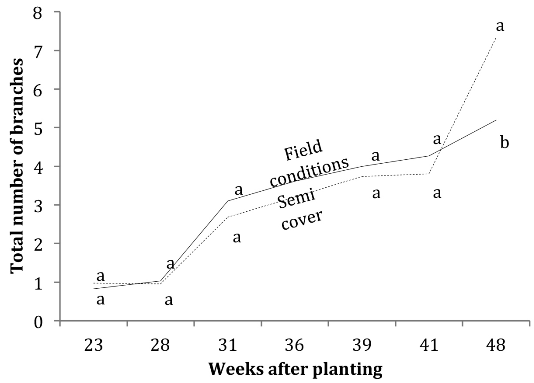

Figure 2. Total number of blackberry branches under the influence of two cropping systems over time.

Averages with the same letter are not statistically different according to Duncan test at 5%. Number of cases, N=200.

Cropping system under field conditions obtained 41% more from total number of branches than semi cover (total number of branches= 7.3). Significant differences (P<0.001) were reported in the number, length, diameter, and flowers of productive branches as well as the percentage of unproductive branches. Cropping system under field condition, following the trend observed in the total number of branches, got a representative value in the number of productive branches (46% higher than semi cover) and the smallest percentage of unproductive branches (Table 1).

Table 1. Agronomic variables of blackberry cv. thornless under the influence of two evaluated cropping systems and PGPR

Cropping systems	Productive branches					Percentage of unproductive branches
	Number	Length (cm)	Diameter (mm)	Clusters	Flowers	
Field conditions	7.03 a	106.46 b	5.85 b	6.21 a	26.16 a	6.1% b
Semi cover	4.82 b	129.86 a	8.25 a	4.96 a	13.15 b	18.6% a
Level of significance	< 0.001	< 0.001	< 0.001	ns	< 0.001	<0.001
Bacterial strains						
Control	6.10 a	127.02 a	7.19 a	7.16 a	26.18 a	13.6% a
GIBI-206	5.73 a	115.19 a	6.91 a	3.67 b	17.17 a	10.9% ab
GIBI-200	5.70 a	108.94 a	6.73 a	3 b	12.33 a	9.4% b
GIBI-200 + GIBI-206	6.29 a	117.85 a	7.25 a	8.29 a	20.74 a	11.1% ab
QST-713	6.18 a	115.89 a	6.65 a	5.72 ab	21.86 a	7.2% b
Level of significance	ns	ns	ns	< 0.01	ns	< 0.05

Averages with the same letter are not statistically different according to Duncan test at 5%.

Number of cases, N=200

In percentage terms, under field conditions system reached a ratio of productive branches of 93.9%, while semi cover system had achieved percentages of 81.4%. In contrast, length and diameter of productive branches was significantly greater in the semi cover system than under field condition system. Average number of clusters per branch did not register significant differences. However, number of flowers per raceme, an important component of yield in this fruit crop, showed an increasing of more than 100% in the cropping system under field conditions compare to semi cover system (Table 1).

Bacterial strains

During samplings performed in weeks 28, 31, and 39 after planting significant differences were observed (P<0.05). In these dates Control treatment (conventional mineral fertilization) showed the highest values in total number of branches (total number of branches in: week 28 = 1.2, week 31= 3.7, and week 39= 4.6) (Figure 3).

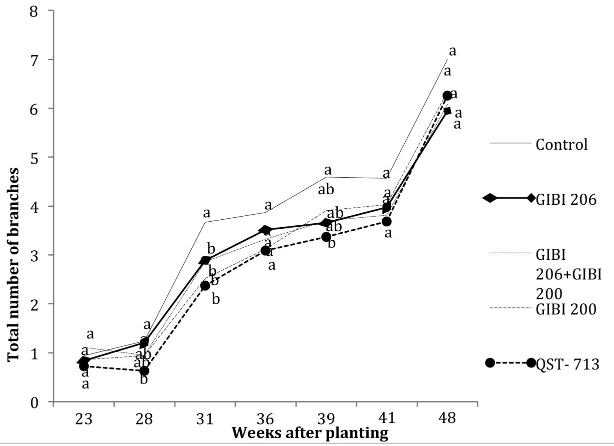

Figure 3. Total number of blackberry branches under the influence of PGPR over time.

Averages with the same letter are not statistically different according to Duncan test at 5%. Number of cases, N=200

However, Duncan test established two groups in weeks 28 and 39, which were composed as follows: *i)* group A: Control, GIBI-206, GIBI-200 and GIBI-200+GIBI-206 and *ii)* group B: QST-713 (only common treatment in both weeks is mentioned). The entire group is explicitly described in Figure 3. In week 31, group A of Duncan test was only composed per control treatment while group B were composed per remaining treatments (common treatments in the groups were not observed). In week 48, the last week, this variable was evaluated, GIBI-200 + GIBI-206 (mixture) and commercial strain QST-713 presented the closest averages in this variable (respectively total number of branches = 6.3 and 6.2) compare to the Control treatment (total number of branches = 7) with no significant differences. Remaining weeks (23, 36, and 41) significant differences among treatments (bacterial strains and control) were not evident (Figure 3).

Additionally, number, length, diameter, and flowers of productive branches of the evaluated strains did not have significant differences, i.e., treatments GIBI-200, GIBI-206, QST-713 and GIBI-200 + GIBI-206 (mixture), obtained similar values to those of conventional management of blackberry cultivation (control) in terms of number, length, diameter and flowers per cluster, however, values of GIBI-200 + GIBI-206 could be highlighted (Table 1).

Alternatively, clusters per branch and percentage of unproductive branches respectively obtained a p value smaller than 0.01 and 0.05. The percentage of unproductive branches exposed the highest values in control treatment (Table 1). Nonetheless, Duncan test was configured in two groups as follows: *i)* group A: Control, GIBI-206, GIBI-200 + GIBI-206 (mixture) and *ii)* group B: GIBI-206, GIBI-200, GIBI-200 + GIBI-206 (mixture), and QST 713. Similar to results obtained in unproductive branches, group A of Duncan test was composed by control, and GIBI-200 + GIBI-206, nevertheless, in this variable QST-713 was part of this group as well as group B. Control and GIBI 200 + GIBI 206 revealed the highest value in number of flowers per cluster, being statistically similar (Table 1).

Discussion

Cropping Systems

Total number of branches over time had achieved a similar behavior to logarithmic phase of sigmoid curve that represents a normal plant growth (Steward, Mapes & Ammirato, 1969). According to Agroclear® plastic film features, semi cover cropping system presented a 15% reduction in total light transmission compare to system under field conditions. This variable (solar radiation) is a decisive factor for plant growth and development. For instance, in crops such as apple (*Malus domestica* Borkh.), plant growth indicators such as leaf area, total number of branches, number of flowers and fruit set, were influenced by shading (Casierra-Posada & Ludders, 2000).

Conversely, López, Marulanda & López (2009), reported values of 98% for productive branches and 2% for non-productive branches in plant materials of *Rubus glaucus* cv. *thornless* in the department of Risaralda, Colombia. These results could be compared with those obtained for cropping system under field conditions. Length of the branches is influenced by the availability of light to plant due to stem sensitivity to auxins, which increases as solar radiation decreases, inducing anatomical changes in plant tissue (Basuk & Maynard, 1987). A continued plant exposure to far-red light (700-800 nm), have allowed an increasing in carbohydrates synthesis addressed to plant stems (Bastías & Corelli-Grappadelli, 2012). In addition, stem diameter could be affected by an increasing in its dimensions under moderate crop shading such as lulo (*Solanum quitoense* Lam.) (Casierra-Posada, Peña-Olmos, Peñaloza & Roveda, 2013).

The number of flowers per cluster is a very important variable in blackberry cultivation due to its direct relation with productivity. Number of flowers per cluster obtained by López, Marulanda & López (2009), in similar climate conditions is 47. However, this study obtained a maximum of 26 flowers both in control treatment and under field conditions system. In experiments carried out with apple (*Malus domestica* Borkh.), induction of flowering was inhibited by plastics of different colors, demonstrating that the quality of solar radiation is an important factor in variables of agronomic relevance in this crop (Solomakhin & Blanke, 2008).

Bacterial strains

Control treatment shows that number of branches increases temporarily equaling with bacterial strains over time. When performing mineral fertilizations, fixation of soil nutrients and their leaching make difficult the presence of these available minerals to plants, which had achieved a decreasing in the fertilization efficiency. In contrast, strains of *B. subtilis* in apple showed that effects on plant growth linked to the mechanisms of action of this PGPR could last up to five years (Utkhede & Smith, 1992). In *Rubus glaucus* cv. thornless, mixtures of rhizospheric microorganisms influence plant growth of *in vitro* seedlings and report best results (Pérez-Moncada, Ramírez-Gómez, Nuñez-Zarante, Franco-Correa & Roveda-Hoyos, 2012).

In commercial blackberry crops, non-productive branches are thin, with a positive geotropic plant growth, and branches are buried in the soil, which facilitates the generation of future productive branches (Zuleta-Ospina, 2014). Conversely, Pérez-Moncada *et al.* (2012), reported that different PGPR and arbuscular mycorrhizal fungi combinations outweigh the impact on the length of blackberry plants under *in vitro* conditions compared to treatment based on 100% mineral fertilization. In addition, *Bacillus subtilis* has shown to have an effect on stem length of sour cherry (*Prunus cerasus* L.) seedlings (Karakurt, Kotan, Dadaşoğlu, Aslantaş & Şahin, 2011) and *Bacillus pumilus* on the stem length and roots of lenscale (*Atriplex lentiformis* (Torr.) S.Watson.) (De-Bashan, Hernandez, Bashan & Maier, 2010). Moreover, in sweet cherry (*Prunus avium* (L.) L.), PGPR improved performance and therefore flowering (Esitken, Pirlak, Turan & Sahin, 2006) and, in apple the effect on yield and harvest, implicitly linked to flowering, may last up to three years under the inoculation of these PGPR (Utkhede & Smith, 1992). In blackberry crops, the number of flowers per cluster is a good crop indicator due to its high fruit set percentage (89%).

Conclusion

Blackberry cv. thornless under semi cover system did not show an increasing in the variables directly related to yield components, only influencing the length and diameter of productive branches. However, semi cover system should be evaluated in future studies as cropping system dedicated to produce plant material for blackberry propagation. Given these concerns, cropping system under field conditions remains the most promising alternative for the commercial development of this cultivar (exclusively focused on yield). On the other hand, PGPR of the genus *Bacillus* demonstrated a representative effect on blackberry plant growth. These effects were similar to mineral fertilization. This fact, can postulate endogenous strains GIBI-200 and GIBI-206 as biofertilizers due to long-term results obtained in this study, which were similar to those correlated with synthetic mineral fertilization. *B. subtilis* GIBI-200 + *B. pumilus* GIBI-206 trend to produce synergetic effects as PGPR. For that reason, new studies evaluating the effect of different PGPR combination in blackberry plant growth are suggested.

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