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Biological, botanical and chemical alternatives for the control of blackberry (*Rubus glaucus* Benth.) diseases

Alternativas biológicas, botánicas y químicas para el control de enfermedades en el cultivo de la mora (*Rubus glaucus* Benth)

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Oscar Darío Hincapié Echeverri^{1*}, Alegría Saldarriaga Cardona², Cipriano Díaz Díez²**ABSTRACT****Keywords:**

Fruit trees
Phytopathology
Antagonistic fungi
Biocontrollers
Integrated management
Organic management

In order to control the main diseases that affect blackberries (*Rubus glaucus* Benth.), a research in which 12 treatments to San Antonio ecotype plants originated *in vitro* was conducted. These treatments were: 1: *Trichoderma harzianum* + *Trichoderma koningii* (Tropical Fungus), 2: *Trichoderma* sp. (Bioprotection), 3: *Trichoderma koningiopsis* (Th003 *Trichoderma*), 4: *Trichoderma asperellum* (Th034 *Trichoderma*), 5: *Trichoderma asperellum* (T-30 *Trichoderma*), 6: *Trichoderma asperellum* (T-98 *Trichoderma*), 7: *Burkholderia cepacia* (Botrycid), 8: Extract of *Swinglea glutinosa* (Ecoswin), 9: Traditional farming treatments (Mancozeb, Propamocarb), 10: Chemical products applications (Mancozeb, Mandipropamida, Carbendazim, Propamocar and Metalaxil+Mancozeb) alternated according to the impact of the disease, 11: Chemical products applications alternated with organic products according to the suppliers recommendations and presence of the diseases, 12: Absolute control, no treatment was applied to the plants. The applications were carried out every 15 days, each plant was an experimental unit and each treatment was made of five experimental units. 12 treatments were made through a RCBD (randomized complete block design) with three repetitions for a total of 15 experimental units per treatment. The assessments were performed every 8 days, and the variables were: number of healthy and sick fruits/treatment, costs/treatment and gross profit. A variance analysis and a Tukey test 5% were made. The best treatments were T11 (rotation of chemicals with biological products), T10 (rotation of chemical products according to the impact of the disease) and T3 (*T. koningiopsis*); considering the obtained performance/treatment, treatment cost and profit.

RESUMEN**Palabras claves:**

Frutales
Fitopatología
Hongos antagonísticos
Biocontroladores
Manejo integrado
Manejo orgánico

Para el manejo de las principales enfermedades de la mora (*Rubus glaucus* Benth.), se realizó una investigación donde se aplicaron 12 tratamientos a plantas ecotipo San Antonio, procedentes de cultivo *in vitro*. Los tratamientos fueron 1: *Trichoderma harzianum*+*Trichoderma koningii* (Hongos del Trópico), 2: *Trichoderma* sp. (Bioprotección), 3: *Trichoderma koningiopsis* (Th003 *Trichoderma*), 4: *Trichoderma asperellum* (Th034 *Trichoderma*), 5: *Trichoderma asperellum* (T-30 *Trichoderma*), 6: *Trichoderma asperellum* (T-98 *Trichoderma*), 7: *Burkholderia cepacia* (Botrycid), 8: Extracto de *Swinglea glutinosa* (Ecoswin), 9: Tratamiento tradicional del agricultor (Mancozeb, Propamocarb), 10: aplicaciones de productos químicos (Mancozeb, Mandipropamida, Carbendazim, Propamocar y Metalaxil+Mancozeb), en rotación según incidencia de las enfermedades, 11: Aplicación de productos químicos en rotación con biológicos, según recomendaciones de los proveedores y presencia de las enfermedades, 12: Testigo absoluto, no se aplicó ningún tratamiento a las plantas. Las aplicaciones se realizaron cada 15 días, cada planta fue una unidad experimental y cada tratamiento estuvo compuesto por cinco unidades experimentales, se realizaron 12 tratamientos en un diseño de BCA con tres repeticiones, para un total de 15 unidades experimentales por tratamiento. Las evaluaciones se realizaron cada ocho días y las variables consideradas fueron: número de frutos sanos y enfermos/tratamiento, costos/tratamiento y ganancia bruta. Se realizó un análisis de varianza y una prueba de Tukey 5%. Los mejores tratamientos fueron el T11 (rotación de productos químicos con biológicos), el tratamiento T10 (rotación de productos químicos según la incidencia de las enfermedades) y el tratamiento T3 (*T. koningiopsis*); considerando los rendimientos obtenidos/tratamiento, el costo del tratamiento y la ganancia alcanzada.

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Mora de Castilla (*Rubus glaucus* Benth.) is affected by diverse diseases mostly caused by fungi, amongst these diseases there is the Anthracnose, which is caused by *Glomerella cingulate* (Stoneman) Spauld. and Schrenk. (anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.), *Colletotrichum acutatum* Simmonds., *Colletotrichum boninense* Moriw., Sato and Tsukib.; these pathogens affect stems, petioles and reproductive structures causing lesions that can end in the death of the plant's branches affecting the crop production (Saldarriaga *et al.*, 2008; Saldarriaga, 2006; Afanador *et al.*, 2009; Tamayo, 2009; Rueda, 2010). The Fungus that causes the Anthracnose can also damage flower buds, tender shoots, stalks and fruits (Franco and Giraldo, 2000). When the fungus infects the plant's side branches, this creates a deformation on the group of flowers, the tender shoots blacken, and the branches die in a descendent and progressive way (Castro and Díaz, 2001). In the inflorescences, this infection appears as a necrosis of the tissues in which the fungus sporulates (Rueda, 2010). In ripen fruits, the disease appears sporadically. In this case, the affected fruits show dehydration, necrosis and wet rot in the presence of some fungal structure (Saldarriaga and Bernal, 2000; Botero *et al.*, 2002). This disease deteriorates between 50 and 70% of the cultivated blackberry plants' stems and causes a loss of a 5% of the pathogens-affected fruits (Tamayo, 2009). The incidence of this disease has been registered in a 52% under the conditions given by the blackberry crops in the Colombian coffee belt region (Botero *et al.*, 2002).

The gray mold caused by *Botrytis cinerea* Pers, Ex. Fr., is another important disease that appears during the stage of production and postharvest of the blackberry. This fungus affects the flower buds since their opening and appears in the fruition and ripening, causing necrosis and mummification of the fruits (Forero de La-Rotta, 2007) (Quinatoa, 2015). In the presence of humidity, the affected fruits show a growth in the fungus with a velvety aspect of colors gray, brown or olive green. The fruits dry and mummify stuck to the cluster (Saldarriaga and Bernal, 2000; Tamayo, 2009). The fungus can also affect leaves, flowers and stalks (Franco and Giraldo, 2000). This disease has created losses between 50 to 76% of the harvested fruit affected by pathogens (Tamayo and Peláez, 2000).

The downy mildew caused by *Peronospora* Corda affects the stems, stalks, flower buds, flowers and fruits. The sick stems and stalks show purple discolorations with whitish lesions on which a downiness of light gray color grows (Saldarriaga and Bernal, 2000; Tamayo, 2003). The flowers show yellowing then their petals get dry to fall later on. The sepals show a light brown to black color lesion which advances until the flower buds mummification (Tamayo, 2009). In fruits, the fungus causes an irregular development, uneven ripening, and loss of fruit brightness, which depreciates the fruit in its commercialization. The green fruits stop growing (Saldarriaga and Bernal, 2000; Franco and Giraldo, 2000). This disease can cause losses of a 20 to 30% of the harvested fruits (Tamayo and Peláez, 2000).

Another disease that can affect the blackberry is the powdery mildew. This is caused by the fungus *Oidium* Link. This infection appears mainly in young leaves through deformation (leaf curling), it is associated to the presence of chlorotic, irregular and diffused areas that can be seen on the surface of the foliar laminae. Occasionally, the leaves are covered on a fine white dust that corresponds to the growth and sporulation of the fungus. The yellow rust is also an important disease; this one is caused by the fungus *Gerwasia lagerheimii* (Magnus) Buritica, it appears in the form of pustules of orange color in the stems, stalks, the reverse of leaves and in the fruits (Franco and Giraldo, 2000; Tamayo, 2009).

There are reports of foliar conditions caused by the fungi *Septoria* Sacc, *Phyllosticta* Pers, *Alternaria* Nees, which importance is still secondary due to the low incidence of the diseases caused by them (Tamayo, 2009). The stems and roots of the blackberry also present some diseases caused by fungi such as: *Coniothyrium fuckelii* Sacc, *Verticillium albo-atrum* Reinke and Berth, *Rosellinia* De Not, *Fusarium roseum* Link: Fr. and *Fusarium oxysporum* Schlechtend. Fr, which importance has not been established yet (Tamayo, 2009).

Traditionally the treatment of the diseases of the *Mora de Castilla* has been based on the development of some cultural practices and the periodic application of different fungicides —there is no official record of most of them being used for the blackberry—. The current tendencies in food production are oriented towards getting products with less pesticide charges, besides

the demands on environmental conservation and food harmlessness. Nowadays, in the field of blackberry harvesting, the use of fungicides is starting to be restricted or forbidden, such as the products based on Clorotalonil, Mancozeb, Propineb, Metalaxil + Mancozeb, Propineb + Cymoxanil (Arroyave and Salazar, sf., quoted by Díaz, 2009). Research institutions and some companies in the commercial sector are developing products of botanical origins such as vegetables extracts, and biological origins such as antagonistic microorganisms with the potential use of the control of the diseases caused by fungi of the genus *Colletotrichum*, *Botrytis*, *Peronospora*, *Oidium* (Restrepo *et al.*, 2001; Agrobiológicos Safer and Natural Control Sf, Vélez and Estrada sf, Bioprotección sf, Ecoflora, 2007; Velosa *et al.*, 2002; 2003, Castro and Díaz, 2001).

This situation has created the need for more research on new products and management strategies that allow the offering to the blackberry producers of more efficient and sustainable alternatives to the integral management of the main diseases that affect this crop, contributing to the obtaining of a product harmless to the consumers. With the results from this research, some key knowledge is provided to the creation of blackberry disease control proposals, considering among others, the efficiency of the products and the economic sustainability to satisfy the demands of the food and agriculture chain of the blackberry.

MATERIALS AND METHODS

The in-field observations were carried out in a period of 20 months in Corpoica R.C. La Selva, Rionegro, Antioquia, under conditions of a Lower Montane moist forest (LM-mf). 12 treatments were applied to blackberry plants of the San Antonio ecotype, originated *in vitro*. The evaluated treatments were the following: T1: *Trichoderma harzianum*+*Trichoderma koningii* (commercial product of *Hongos del Trópico*), T2: *Trichoderma* sp. (comercial product of *Bioprotección*), T3: *Trichoderma koningiopsis* (Th003 *Trichoderma*, product of CORPOICA), T4: *Trichoderma asperellum* (Th034 *Trichoderma*, product of CORPOICA), T5: *Trichoderma asperellum* (T-30 *Trichoderma*, product of the Corporación para Investigaciones Biológicas – CIB, in scaling process), T6: *Trichoderma asperellum* (T-98 *Trichoderma*, product of the CIB, in scaling process), T7: *Burkholderia cepacia* (Botrycid, commercial product of *Safer*), T8: extract of *Swinglea glutinosa* (EcoSwing® commercial product of

Ecoflora), T9: corresponds to the traditional treatments of the farmer based on the applications of chemical products with active ingredients such as (Mancozeb) and (Propamocarb), T10: Application of chemical products (the products with the following active ingredients were applied: (Mancozeb), (Mandipropamida), (Carbendazim), (Propamocarb), (Metalaxil+Mancozeb) alternating according the effect and incidence of the blackberry diseases, T11: Application of chemical products alternating with biological products (bio-control microorganisms and vegetal extracts) according to the recommendations of the suppliers and the phenological state of the plants; the following products were applied: extract of *Swinglea glutinosa*, *Burkholderia cepacia*, *Trichoderma koningiopsis* (Th003), *Trichoderma asperellum* (T-98), *Trichoderma* sp., Propamocarb, Metalaxil +Mancozeb, Mandipropamida, taking into account the presence and incidence of the diseases, T12: Absolute control, no treatment was applied to the plants. The 12 treatments were distributed in three completely randomized blocks, each plant was an experimental unit and each treatment was made of five experimental units.

The plants that underwent the treatment were surrounded by blackberry plant sources of inoculum, for a total of 270 plants sown with a distance of 2 m x 2 m between plants and 3 m x 3 m between furrows, in an area of 1530 m² (Díaz, 2009; Hincapié, 2010). Previous to the crops' establishment, a chemical analysis of the soil was made which was used as a base to the fertilization process. The cultural labors proper to the crops were made such as the *plateo* (keeping the young plants clear of weeds), weed control, pruning, wiring on a modified double T trellis system, maintenance pruning (leaving 5 stems per plant), sanitary pruning, and destruction of the pruning remainders. The plot was monitored on a weekly basis and the application of the treatments started once a 50% of the plants were blossomed. These applications were carried out every 15 days, using the doses recommended by the products manufacturers and traders. The evaluations were carried out every eight days, by collecting and separating the healthy fruits from the sick on each type of treatment in order to be quantified in the laboratory later on through the processes of counting, weighing, classification and registry of the fruits affected by the diseases. The following were taken into consideration: the amount of healthy fruits

and their weight, the number of fruits affected by the gray mold (*Botrytis cinerea*), downy mildew or peronospora (*Peronospora* sp.), and the anthracnose (*Colletotrichum* sp.). For each of the treatments the performance per treatment (kg ha^{-1}) was estimated. For the mentioned variables, an analysis of variance and the medians separation according to the Tukey test on a 5% level were conducted. Besides,

the cost of the different treatments was calculated and the gross profit was estimated per hectare sown (1666 plants). The experiment was suspended once the plants reached the 11 month of the productive stage (the peak or production under the agro-ecological conditions where the plot was established is reached 18 months after the beginning of the productive stage) (Díaz, 2009; Hincapié, 2010).

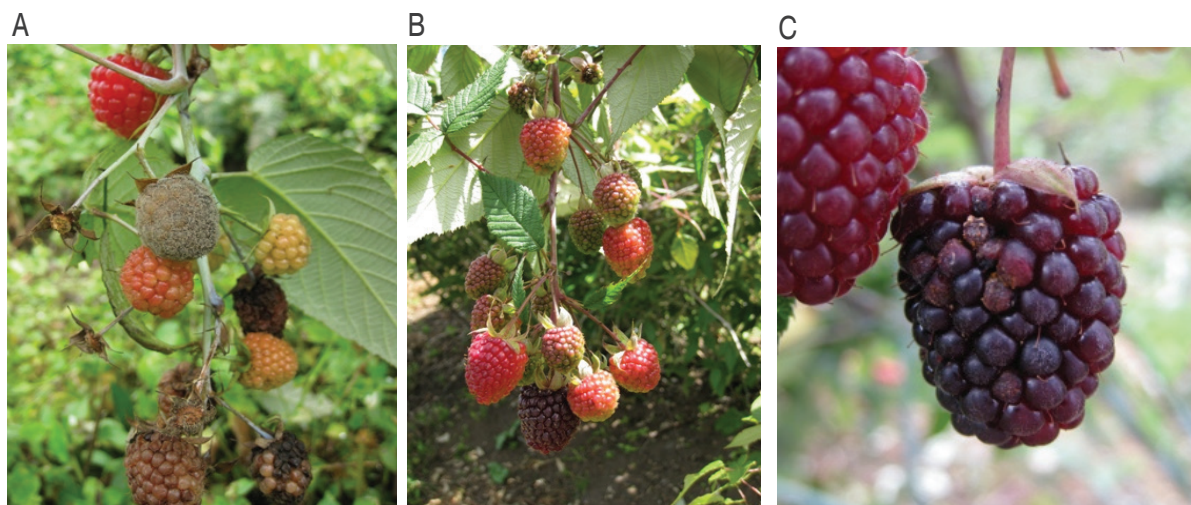


Figure 1. A. Blackberry fruits affected by gray mold (*Botrytis cinerea*). B. Blackberry fruits affected by downy mildew (*Peronospora* sp.). C. Blackberry fruits affected by Anthracnose (*Colletotrichum* sp.).

RESULTS AND DISCUSSION

The results obtained through this research are the following: on the Absolute Control treatment (T12), the treatments that showed a higher percentage of healthy fruits, with no significant statistical differences, by the gray mold, downy mildew and Anthracnose diseases (Figure 1) were: T11, T10, T3 and T9 (Table 1). For this group of treatments, the percentage of sick fruits was between 35 and 39%; whereas the Absolute Control group presented an 81% of sick fruits. In this same group of treatments, the best results for the gray mold management were achieved with the use of T3 and T11 (with percentages between the 2.2 and 2.5%) in contrast to the Control group that presented a 15%. For the downy mildew the percentage of sick fruits was between the 32.3 and 36%, being the best treatments the T10, T11 and T9 in contrast to the Control group which presented a 64.2% (Table 1). Regarding the Anthracnose, the percentage of affected fruits was less than 2.1%. Treatment T8 did not show any difference from the Absolute Control

T12, but both presented differences from the rest of treatments (Table 1).

In addition to this, after analyzing all treatments, and independently the behavior of the diseases, the following was observed: for the gray mold the best treatments were the T1, T3 and T11 which presented the lowest percentage of fruits affected by the disease (2.2% to 2.5%) (Table 1); to this group the treatments that followed are T7, T5, T10, T4 and T8 with percentages between the 3 and 4% of the fruits affected by the disease with no significant differences between them. Treatments T6, T9 and T12 were the less efficient. Treatment T12 showed significant differences from all treatments and reached the highest percentage of fruits affected by *Botrytis cinerea*, corresponding to a 15%. Figure 1A illustrated the symptoms of the fruit affected by gray mold, these are consistent to a soft rot in which appears a growth of grayish color that corresponds to the sporulation of the fungus.

Table 1. Behavior of the different treatments according to the percentage of healthy and sick fruits.

Treatments	Healthy fruits (%)	Sick fruits (%)	Fruits with gray mold (%)	Fruits with downy mildew (%)	Fruits with anthracnose (%)
T1	48.69 b	51.31 b	2.27 d	46.19 bc	0.71 bc
T2	46.70 b	53.09 b	5.64 b	46.44 bc	1.05 bc
T3	61.02 a	39.00 c	2.28 d	36.00 c	0.71 bc
T4	49.90 b	50.10 b	4.18 bc	44.73 bc	1.19 b
T5	42.26 b	53.74 b	3.84 bc	49.05 b	0.85 bc
T6	41.92 b	58.08 b	4.81 b	52.14 b	1.16 b
T7	42.68 b	57.36 b	3.74 bc	52.76 b	0.95 bc
T8	44.86 b	55.14 b	4.24 bc	48.80 b	2.10 a
T9	60.62 a	39.38 c	5.00 b	33.89 d	0.58 bc
T10	63.07 a	36.93 c	3.95 bc	32.35 d	0.64 bc
T11	64.34 a	35.66 c	2.56 cd	32.37 d	0.74 bc
T12	19.00 c	81.00 a	15.04 a	64.24 a	1.72 a
Variance coefficient	11.47%	11.06%	20.69%	11.33%	44.78%

Results with equal letters have no significant difference between treatments.

Regarding the downy mildew, this was the disease that showed the high percentages of affected fruits (32% to 64%). With treatments T10, T11, T9, the lowest percentages of sick fruits were obtained, around 32% (Table 1). For the other treatments the percentage of sick fruits was between the 36 and 52.7%. Some significant differences were established with the Absolute Control group, which reach a 64% of fruits with symptoms of the disease. This disease manifests with bad filled drupes, uneven ripening, irregular development (Figure 1B), and the fruits harden when touched.

Regarding the Anthracnose, this disease showed the lower percentage of affected fruits (less than 2.1%) Other studies have reported losses by Anthracnose estimated in approximately a 5% of the fruits affected by different diseases (Tamayo, 2009). In the fruits the disease appears sporadically and the symptoms correspond to drupes dehydration, necrosis (Figure

1C), and wet rot with presence of fungal structures (Saldarriaga and Bernal, 2000).

During this study, besides the behavior of the diseases in front of the different treatments, the production (kg of healthy fruits per treatment ha⁻¹), the production costs (assuming \$2000 COP as the sale value of a kilo of blackberry), and the cost of the treatment were considered. Table 2 registers the respective values and costs for each of the treatments.

The highest production (t ha⁻¹ per year) was reached on treatments T10, T9 and T11; however, when the costs analysis was made, it showed that the value of consumables for some treatments was considerable and affected negatively the profit; leading in some moments to produce losses, like can be seen on treatment T9.

T11 (chemical products alternating with biological products and vegetal extract) was considered as a promising

Table 2. Costs and gross profit for every treatment.

Treatment	Performance (t ha ⁻¹ year ⁻¹)	Production value (\$cop)	Cost of treatment (\$cop)	Gross profit
T1	2.50	4,992,737	12,856,168	-7,863,431
T2	2.31	4,626,915	1,455,272	3,171,643
T3	2.68	5,365,805	121,285	5,244,520
T4	2.43	4,858,596	121,285	4,737,311
T5	2.30	4,595,323	621,973	3,973,350
T6	2.21	4,413,292	621,973	3,791,319
T7	1.91	3,819,759	5,146,826	-1,327,067
T8	1.85	3,691,563	8,816,472	-5,124,909
T9	7.24	14,482,753	15,001,997	-519,244
T10	8.25	16,507,466	8,915,720	7,591,746
T11	5.54	11,076,267	5,206,295	5,869,972
T12	1.26	2,517,418	0	2,517,418

treatment due to the gross profit and the possibility of reducing the charge of chemical products and the addition of biological products, which can contribute to the environmental conservation and the obtaining of a harmless product. In order to apply this treatment it's important to monitor the diseases regarding their happening and severity to define the type of product that will be used, besides the weather conditions at the moment of application have to be considered. Treatment T10 (chemical products rotation according to the happening of diseases) allowed to obtain the highest production and gross profit (Table 2), and a most rational management of the diseases but it needs the implementation of a monitoring component of the diseases as a selection criteria of the products to apply. Treatment T3 (Th003 - *T. koningiopsis*) can be an alternative to contribute to the integrated management especially in crops with a high incidence of gray mold. Currently, the laboratory of biological control of Corpoica has a wet powder (WP) product made from *Trichoderma koningiopsis* Th003 named "Tricotec". This bioinsecticide has demonstrated excellent results in vegetable crops for the pathogens

control such as *Rhizoctonia solani*, getting a reduction of the disease of a 71% in Chonto tomato (Gómez *et al.*, 2011; Gómez, 2012). It has also shown a high efficacy in the control of *Botrytis cinerea* and *Oidium lycopersicum* in tomato by being applied to the phyllosphere; nevertheless, studies on the valuation of technology indicated a high economic feasibility for the soil application and the foliar application (Gómez *et al.*, 2011). According to Gómez (2012), in the trials made on lettuce with the bioinsecticide (WP) *T. koningiopsis* Th003 symptoms of the pathogen *Botrytis* spp. did not appear.

CONCLUSIONS

The best results were obtained through the following treatments: T11 (use of chemical products alternating with biological products —biocontrollers and botanic extracts—) followed by treatments T10 (chemical rotation of products according to the happening of diseases), and the treatment T3 made of *Trichoderma koningiopsis* (Th003); all of this considering the obtained performance/ treatment, the cost of each treatment and the gross profit among others.

The treatments evaluated under experimental conditions were inefficient for the control of the downy mildew (*Peronospora* sp.), getting better results on the control of the gray mold and the Anthracnose of the fruit when said treatments are applied combining the opportune and adequate implementation of the cultural labors (sanitary and ventilation pruning, harvest and destruction of sick fruits, weed control, fertilization and management of pruning residues).

The products' evaluation on different grades of scaling does not allow to make the best comparison due to the possibility of having given more advantages to certain products in relation to other products that were on inferior stages of manufacture.

The low performance of the crops are explained considering that experimentally the work was carried out with only five stems per plant, and besides, the crops corresponded to the beginning of the plants' productive stage and did not reach the peak of the harvest.

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