

Characterization of Anthracnose resistance caused by *Glomerella cingulata* and productivity of five Andean blackberry genotypes (*Rubus glaucus* Benth.)

Caracterización de la resistencia a la antracnosis causada por *Glomerella cingulata* y productividad de cinco genotipos de mora (*Rubus glaucus* Benth.)

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Abstract

The research aimed to evaluate promising materials having both resistance to Anthracnose and high productivity. The experiment was established at the Betania village, municipality of Guática, department of Risaralda, located to 2160 MASL. Five blackberry genotypes previously selected by the research group on Biodiversity and Biotechnology of the Universidad Tecnológica de Pereira, Colombia. Ten months after establishment of the experiment, the plants were inoculated with two pathogenic isolates of *Colletotrichum gloeosporioides* at a concentration of 1.2×10^6 conidia ml⁻¹ of water. The response variables were incidence (presence or absence of injuries on the stems), severity (using a scale of 1 to 9) and yield (kg per plant). The five genotypes tested were classified into three groups according to their resistance to the pathogen and its performance: the first, formed by UTP-7 and UTP-8, which produced 21 and 17.6 t ha⁻¹ respectively; the second, by UTP-1 which produced 14.3 t ha⁻¹, and the third, by UTP-2 and UTP-4 with 9.8 and 7.9 t ha⁻¹, respectively.

Key words: *Colletotrichum gloeosporioides*, disease resistance, epidemiology, fungus, genotypes.

Resumen

En la vereda Betania, municipio de Guática, Risaralda (Colombia) a 2160 m.s.n.m., se evaluaron cinco materiales promisorios de mora (*Rubus glaucus* Benth.) por su resistencia a la antracnosis (*Colletotrichum gloeosporioides*) y por su alta productividad, los cuales fueron previamente seleccionados por el grupo de Investigación en Biodiversidad y Biotecnología de la Universidad Tecnológica de Pereira. Diez meses después del establecimiento las plantas fueron inoculadas con diferentes cepas del hongo a una concentración de 1.2×10^6 conidios/ml de agua. Las variables evaluadas fueron incidencia (presencia o ausencia de lesiones en tallo), severidad (según escala de 1 a 9) y rendimiento (kg/planta). Los genotipos se clasificaron en tres grupos según su resistencia al patógeno y por su rendimiento: el primero, lo conformaron UTP-8 y UTP-7, los cuales produjeron 21 y 17.6 t/ha, respectivamente; el segundo grupo estuvo formado por el genotipo UTP-1 el cual produjo 14.3 t/ha, y el tercero por UTP-2 y UTP-4 con 9.8 y 7.9 t/ha, respectivamente.

Palabras clave: *Colletotrichum gloeosporioides*, epidemiología, genotipos, hongo, resistencia a la enfermedad.

Introduction

In Colombia, the blackberry (*Rubus glaucus* Benth.) is the main source of economical inputs for 6000 small and medium farmers located mainly in the Andean region (Agronet, 2010). It is considered as one of the fruits with larger development potential by its impact in the generation of direct and indirect employments and, it is one of the most important fruits in the crop diversification programs, rural employment offer, food offer and commercial agroindustry as exotic product (Franco and Giraldo, 2000).

According to data of the Ministry of Agriculture and Rural Development (MADR), blackberry production changed from 22,476 t/year in 1992 to 99,182 t/year in 2010; in the same period the sown area increased from 3167 ha to 12,203 ha. The yield has stayed around 8.1 t/ha in the last 18 years, however, in the departments with high production (Santander and Casanare) yields around 15 t/ha have been reached (Agronet, 2010).

Data show a significant increase in the blackberry sown area in the Coffee Area, where were sown 463 ha in 2006 to 845 ha in 2010, however, yields have not increased and, to the opposite, have decreased from 8 t/ha in 2006 to 7.8 t/ha in 2010, being the department of Quindío the most affected with 3.5 t/ha, which means a wide range of unsatisfied demand in pre-harvesting processes of this fruit (Agronet, 2010).

The low yield of blackberry in the country is in large part a consequence of antracnose, disease caused by *Glomerella cingulata* (Stoneman) Spauld and H. Schrenk (Forero de La-Rotta *et al.*, 2002). In Castilla blackberry this disease is presented as dark spots in stems and branches, in which the interior looks gray with dark border before the reproductive structures of the pathogen are developed as conidia masses in salmon color (Tamayo, 2003). One of the most fre-

quent symptoms observed in branches or stems that have been cut, places where the microorganism can penetrate, cause lesions of dark color and define border that rapidly colonize all the tissue causing, in the first case, the descendant death of the branch and in the second, the death of the stem from the base till the superior branches (Forero de La-Rotta, 2001).

When the crop is established in zones of high humidity, the disease attacks the base of the stem and, as difference with other fruit trees, the pathogen is not frequent in floral and reproductive structures (Tamayo, 2003). The more lignified tissues are the most susceptible for the development of the disease which can affect between 50 and 70% of the plant stems (Forero de La-Rotta, 2001). This disease is considered as the most limiting in Colombia for the blackberry crop, because it causes more than 50% of losses and in some cases it can be the total loss of the crop, when preventive measures are not applied (Saldarriaga *et al.*, 2008).

According to the above, it is necessary to look for productive alternatives by collecting, characterizing and preserving the genetic resources that guarantee the development of programs to release blackberry genotypes with high productive potential, heterogeneity, disease adaptation and resistance, supplemented by cloning of selected genotypes. The above is needed in order to improve the competitively the Colombian fruit sector as strategy to position the product in new international markets.

Taking into account the above, the objectives of the present research were: (1) to determine the incubation period of the fungus; (2) measure the incidence, severity, development rate and area under the curve of the disease progress; (3) determine the effect of the weather conditions on the incidence and severity of the disease and; (4) quantify the production and percentage of loss due to the pathogen on each of the

evaluated genotypes.

Materials and methods

The research was done in the village Betania, San Clemente, municipality of Guática, department of Risaralda (Colombia), at the east of the Weastern chain of mountains at 5° 20' 26, 2" N and 75° 47' 28" WO, and 2160 MASL. the experimental area was 3000 m², with undulated topography, 5% slope, previously used for kikuyu grass (*Pennisetum clandestinum*). Weather data were register on a weather station in the field at 1.5 m high which contained a thermohydrograph Thies clima (Intelpro S.A.). The daily accumulated rainfall was measured with a pluviometer Lhaura (Weather, Colombia) with numerical scale placed at 1.5 m above the soil.

In the study five blackberry genotypes were evaluated, they were previously selected as promising in controlled laboratory conditions by Morales *et al.* (2010), which were identified by their genetic and morphological characteristics. In Table 1 are shown the characteristics and the responses of these genotypes to inoculations with *C. gloeosporioides*. To ensure their homogeneity, the genotypes planted in the field were obtained and characterized by *in vitro* propagation protocols for Castilla blackberry, developed by the Plant Biotechnology lab at the Universidad Tecnológica de Pereira (Marulanda *et al.*, 2000). Plants were grown in the greenhouse for 5 months under adequate irrigation and fertilization

conditions before taking them to the field.

A completely randomized block design with four replicates was used, being the experimental unit 15 plants that composed each block. The useful size for the experimental plot was 77.5 m x 34 m for a total of 2635 m² with 527 plants. Plants were sown in August 2009, with a distance between them of 2.5 m and between rows 2 m.

Due to the low tradition of agricultural crops in the area and the scarce blackberry commercial crops close to the place of study, there are low probabilities of natural infestation with the natural pathogen. Three inoculations were done every 15 days between June and July 2010, which was 10 months after the genotypes were established in the field, using two highly pathogenic isolates of *C. gloeosporioides* (3s1 and 6 strains), previously characterized by Morales *et al.* (2010). The inoculation was done by puncture using a hypodermic needle doing 12 wounds in the inferior third of the stem, on them was directly sprayed the spore suspension at a concentration of 1.2×10^6 conidia/ml trying to completely cover the stem. In total were done three inoculations: first one with the 3s1 isolate, second one with the 6 isolate and third one with a mix of both using a mechanical sprayer Easy-to (Fercon) at a dose of 100 cc/plant. Inoculations were only done on the UTP-4 genotype which is highly susceptible to anthracnose and, which was placed as a boundary around all the evaluated plots to ensure the pathogen presence in the field and the inoculum pressure on the genotypes.

Table 1. Blackberry genotypes included in the study.

Field code	Phenotypic characteristic	Response to <i>C. gloeosporioides</i>
UTP-1	Without thorns	Moderately resistant
UTP-2	With thorns	Moderately resistant
UTP-4	With thorns	Highly susceptible
UTP-7	Without thorns	Moderately resistant
UTP-8	Without thorns	Moderately resistant

Source: Morales *et al.*, 2010.

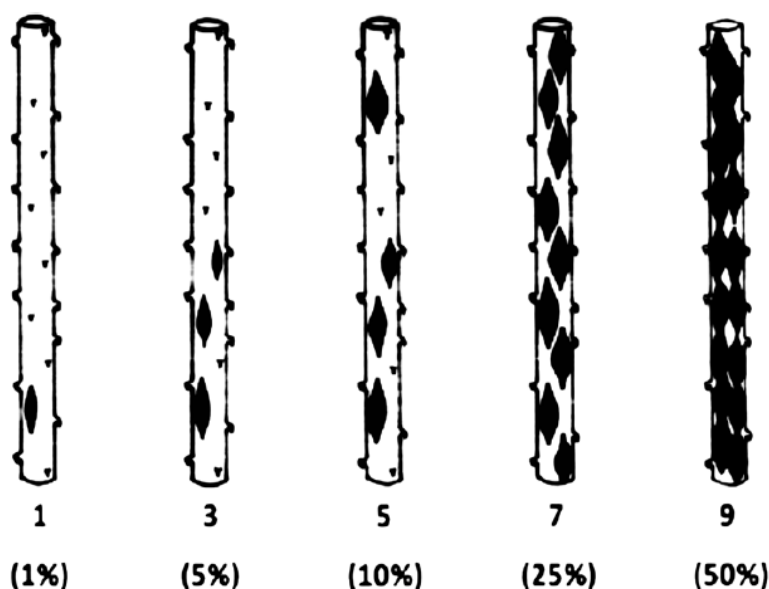
The incubation period of the fungus was determined as the difference in days between the previous inoculation with the pathogen and the presence of the first disease symptoms in the plant stems, according to the methodology of Castaño-Zapata (2002). Finally, each week and during 30 weeks (September 2010 to March 2011), evaluations of the incidence of the disease were done. For that, ten random stems were labeled in each one of the 15 plants per block evaluating the presence or not of the typical lesions of the disease. In each plant was determined the number of healthy and affected stems by the fungus. The incidence index was established by the methodology proposed by Castaño-Zapata (2002).

Together with the incidence evaluations, severity index was determined in two randomly selected stems in each one of the 15 plants per block. Disease severity was determined with a standard diagram according to the methodology proposed by Castaño-Zapata *et al.* (1997) (Picture 1). Readings were stopped when the susceptible genotype (UTP-4) presented 50% or more severity.

The record of the production of fresh

fruit per plant in each block was taken weekly. This record was done from the moment of the first inoculation, June 30, 2010 till March 30, 2011, date of the last disease evaluation. The percentages of yield loss due to the disease were calculated by the difference of each genotype, in comparison with the yield of the best genotype. To estimate the effect on yield reduction due to the severity index, the correlations between both parameters in each one of the evaluated genotypes were calculated.

The crop maintenance consisted on vine arbor and tutoring of the productive branches and in formation pruning to stimulate high budding. Sanitary pruning or specific fungicide applications against *C. gloeosporioides* were not done in order to allow the establishment of the disease in the field. The disease management as gray mold and powdery mildew caused by *Botrytis cinerea* and *Oidium* spp., respectively, was focused to the preventive control with applications of protective products as colloidal sulfur (Azuco® - Agro S.A.) and copper compounds (copper oxychloride 58.8% W.P® - Proficol). The weed control was done each 15 days using scythe and machete for the area surro-



Picture 1. Standard diagram of antracnose severity in blackberry (own authorship).

unding the plant. Fertilization plan was done according to the soil analysis done before the crop establishment.

The data obtained after 30 weeks of evaluation of fungal incidence and severity, as the production, were subjected to simple analysis of variance (Anova) using the statistical software *Statistical Analysis System* (SAS). When significant differences were detected ($P < 0.05$) a Tukey's mean comparison test was performed.

Results and discussion

The manifestation of the characteristic symptoms of antracnoses was observed at 42 days after the last pathogen inoculation. The initial injuries were presented as small circular spots placed mainly on the thorns at the stem base. With disease progress the injuries were observed in the thorns closer to the initial injury changing to gray color with purple edges. Later, the highly affected

thorns were detached which contained acervuli and conidial masses of salmon color (Picture 2).

The time of the starting of the disease (42 days) agrees with the results of Saldarriaga *et al.* (2008) who found that the first disease symptoms appeared around day 39; between days 50 and 75 the chlorosis in the branch is observed and; between the day 80 and 90 causes the death of the branches. In the evaluated genotypes there were not observed symptoms of chlorosis in the leaves or plant death. The disease injuries were only observed on the genotypes UTP-2 and UTP-4, with an incidence of 99% and 80% and a severity of 50% and 46%, respectively (Table 2).

The severity records allowed building of the disease development curve where in the last week is observed that the susceptible control (UTP-4) presented 50% of severity (Figure 1). Only the UTP-2 and UTP-4 genotypes showed disease symptoms with inci-



Picture 2. Typical symptoms of anthracnose on blackberry.

Table 2. Severity of the anthracnose attack on the blackberry evaluated genotypes.

Genotypes	(weeks after incubation period)					
	5	10	15	20	25	30
	Severity (%)					
UTP-1	0.0	0.0	0.0	0.0	0.0	0.0
UTP-2	1.3	2.6	6.1	12.6	23.9	45.6
UTP-4	3.6	5.9	13.1	22.2	41.4	50
UTP-7	0.0	0.0	0.0	0.0	0.0	0.0
UTP-8	0.0	0.0	0.0	0.0	0.0	0.0

dence 99% and 80% and severity 50% and 46%, equivalents to a developmental rate of 0.17 and 0.13 and areas under the curve of disease progress 1039.78 and 861.78 units, respectively ($P < 0.05$).

In general, strong longitudinal breaks were observed in stems, besides the loss of highly sporulated thorns, but in any case, including the strongly affected phenotypes, it was observed generalized death symptoms; that according to Forero de La-Rotta (2001) happens because of a vascular clogging caused by the fungus or rings in the stem of affected areas. As response to the pathogens, the blackberry genotypes grow buds and/or increase the diameter of affected stems causing breaks in the tissues. According to (2003) this reaction happens to counteract the damage in the stem and create new conductive channels; however, this hypothesis has not been demonstrated yet in blackberry plants with advance disease.

During the 30 experimental weeks, the temperature in the field changed between 14.9 and 24.1 °C, with average 19.5 °C. In the last 7 weeks the temperature varied ± 2

°C (Figure 2A). This condition matched the high severity index of the disease. Thus, when the temperature stayed constant, the severity was lower than 20% in both susceptible genotypes; whereas, after week 16, temperature changed from 18.5 to 20.5 °C, matching the highest severity in the genotypes UTP-4 and UTP-2. Forero de La-Rotta *et al.* (2002) in epidemiological studies of antracnose in blackberry, found that when temperature is lower or equal to 13 °C the amount of affected tissue is considerably reduced but, when the temperature is around 16 °C the progress of the injury is higher. Leandro *et al.* (2003) made a similar observation when evaluating the relationship between temperature and length of the humidity on conidia germination, secondary formation of conidia and formation of appressorium of *C. acutatum* in asymptomatic leaves of strawberry. In this case, they observed that under continuous humidity, the optimum temperature for conidia germination was 23 °C, while for appressorium formation it changed between 17.5 and 23 °C and the secondary formation of conidia changed between 21 to 25 °C.

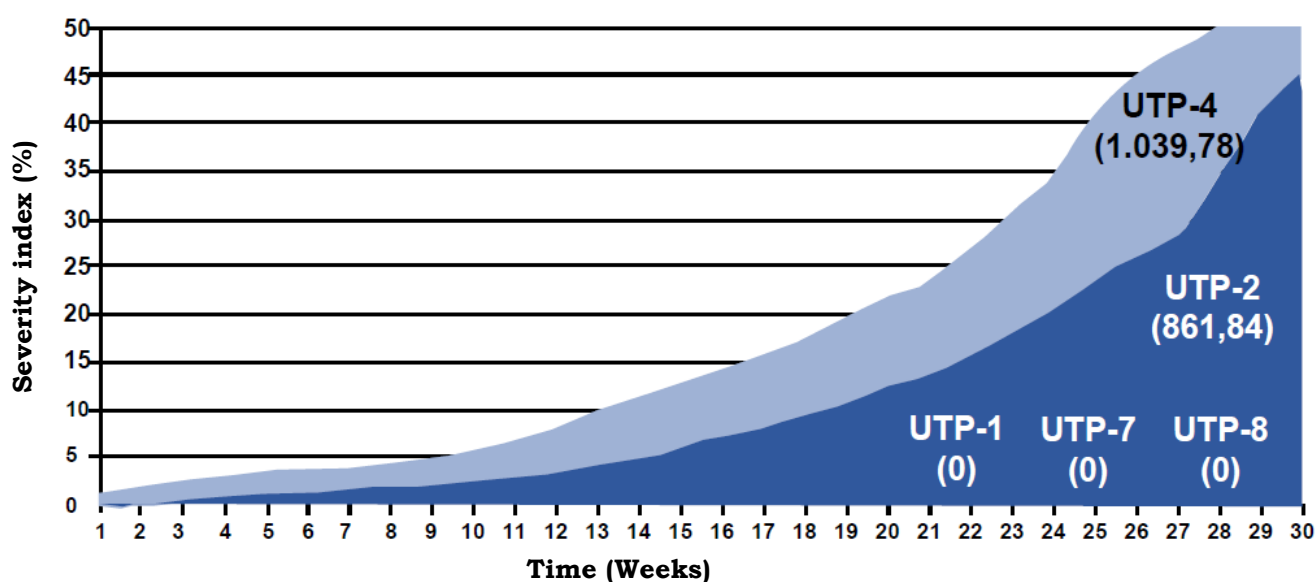


Figure 1. Growth curves and areas under the curve of disease progression based on severity index.

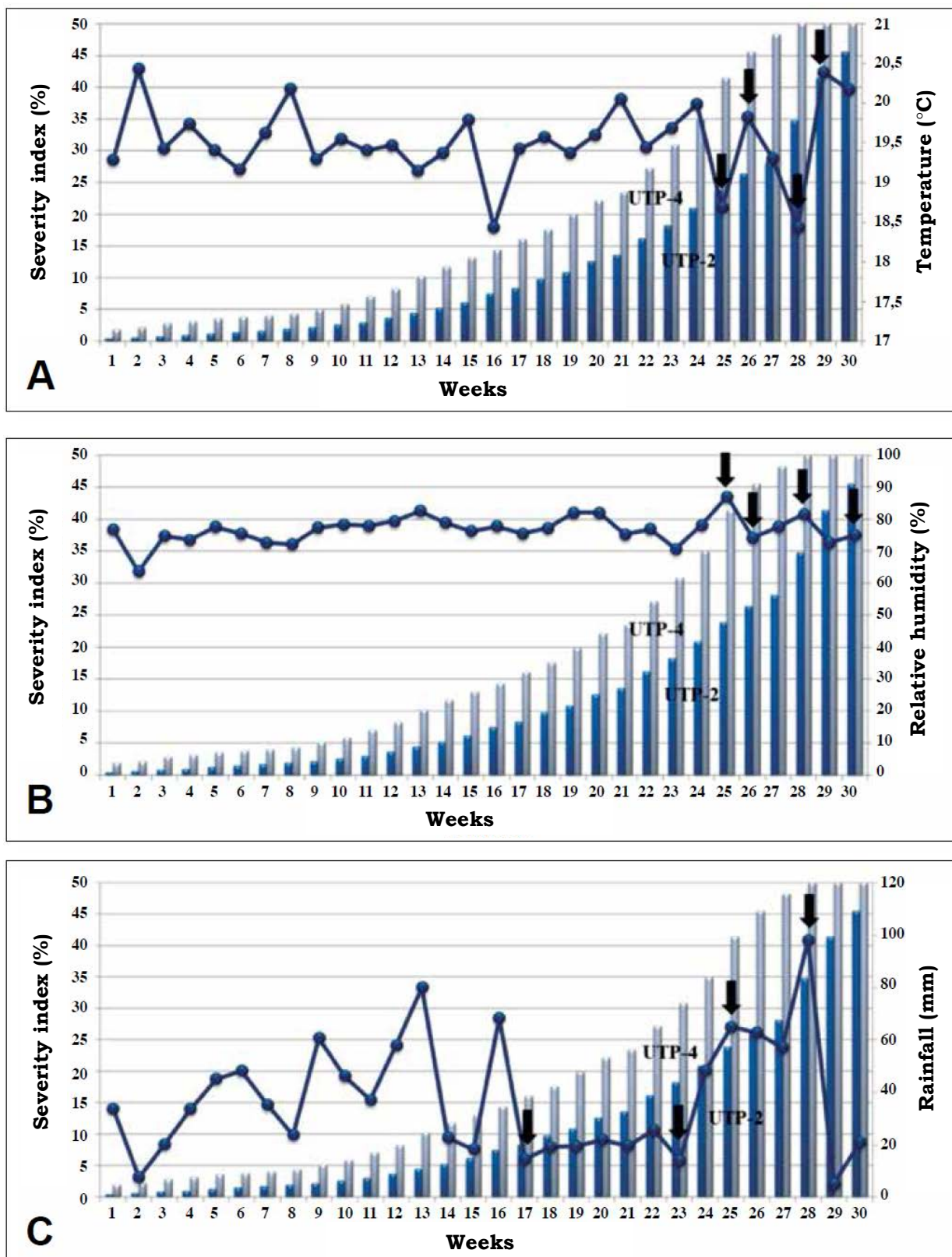


Figure 2. Relationship between severity index and the distribution of the weather conditions during the experimental period (September 8, 2010 to March 30, 2011). **A.** Record of temperature; **B.** Record of relative humidity and **C.** Record of precipitation. The dynamics of each weather factor with the severity index is indicated by arrows.

In this study, the average relative humidity during the evaluation season was 76.34%, presenting the lowest value (63.6%) in week 2 and the highest (87%) in week 25 (Figure 2B). Forero de La-Rotta *et al.* (2002) and Leandro *et al.* (2003) found higher conidia germination of *C. gloeosporioides* and *C. acutatum* among the same time and humidity range, which contrast with the results presented here in which there was no interaction found between disease development and environmental humidity percentage.

Rainfall distribution in the experimental site was variable, showing fluctuations during the 30 weeks (Figure 2C). In total, the accumulated rainfall was 1588.7 mm, with an average of 39.71 mm/week. The week with the lowest rainfall record was week 29 (March 23, 2011) with 5 mm and the highest was in week 28 (March 16, 2011) with 98.2 mm of accumulated rainfall. When associating the rainfall with the severity index average in the susceptible genotypes, it was found that it was not favored when the weekly rainfall was equal or more than 20 mm but, it had an effect when it was more than 25 mm showing an exponential increment. These results agree with the ones of Álvarez (1996), Páez and Zuluaga (1998) and Forero de La-Rotta *et al.* (2002) who estimated that rainfall lower than 19 mm per week do not help the development of the injury but, when rainfall is higher than 35 mm there is a notable increase of the injury.

The influence of precipitation on the pathogen dispersion was studied by Nair *et al.* (1983) and Yang *et al.* (1992) who identified two clear dispersion methods. The first one, although less common, is associated with the ascospores of *Glomerella cingulata*, placed in the harvesting residues or soil as perithecia in latent stage waiting for good conditions to initiate the infection process, in this case, rainfall and wind were the responsible of the ascospore dissemination to the healthy stems. The second method, very common, is associated with the *C. gloeosporioides* conidia which are placed in the acervuli

on the thorns of the plant on a radial way in infected stems, place from which the rain can disperse them to healthy stems.

In Figure 3 is observed the behavior of the yield of each one of the evaluated genotypes. During the first weeks of harvesting, which correspond to the pathogen inoculation time, genotypes had an average yield of 334.5 g/plant, which was reduced to 234.4 g/plant in the third week and to 74 g/plant in the fifth week. This production, that was homogeneous initially, was null between the eighth and eleventh week and notably different between the genotypes as the second harvesting cycle started, after the eleventh week. From the second cycle on, at week 21 (November 17, 2010), time when symptoms of the disease had been already confirmed, yield of the genotypes was variable ($P < 0.05$) (Table 4).

The genotype with higher weekly yield (g/plant) was UTP-8 (906.5), followed by the genotypes UTP-7 (859), UTP-1 (732.5), UTP-2 (550.5) and UTP-4 (452). In the same order, the genotype with higher equivalent accumulated yield (kg/ha) was UTP-8 (21,001), followed by UTP-7 (17,624), UTP-1 (14,387), UTP-2 (9,841) and UTP-4 (7,925).

The genotype UTP-8 is a material of high genetic value due to its high yield that widely exceeds the national and departmental yields of the crop, which, according to Agronet data for 2010 was 8100 kg/ha/year in the first case and 9700 kg/ha/year in the second case for the departments of Risaralda. Franco and Giraldo (2000) considered that a technicized crop, in optimal weather and good resistance to antracnose, could produce between 18,000 and 20,000 kg/ha/year.

The average yield and severity index (5, 10, 15 and 20%) of the susceptible genotypes (UTP-4 and UTP-2) showed a high correlation with coefficient of determination $R^2 = 0.88$ adjusted to the model: $y = 0.237x^2 - 15.619x + 231.9$, where y is equal to the yield loss on a susceptible genotype and x

is the value of an evaluated severity index (Figure 4).

When x values of the logarithmic correlation model were simulated with the

evaluated severity indexes (5, 10, 15 and 20%), the losses were 26, 54, 77 and 93% respectively, which agrees with studies made by Saldarriaga *et al.* (2008), who indi-

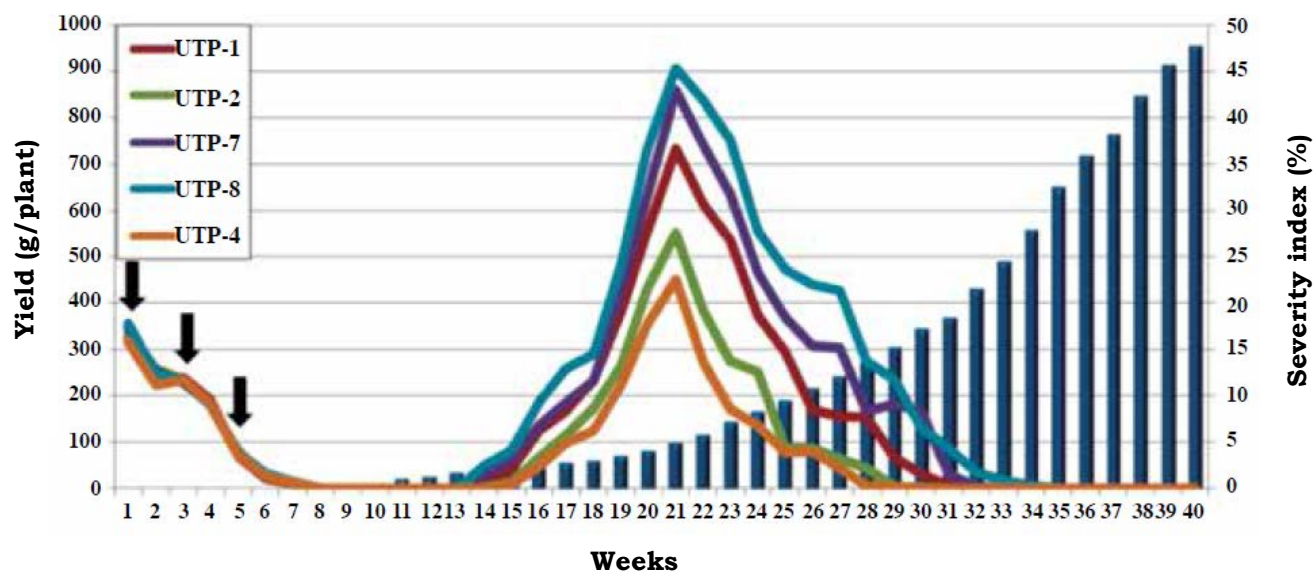


Figure 3. Relationship between the average of the severity index of the UTP-4 and UTP-2 genotypes and the distribution of production in each one of the five genotypes under a constant inoculum pressure. The arrows indicate the inoculation dates.

Table 3. Incidence and severity indexes, areas under the disease progression curve and rates of antracnose development in the evaluated blackberry genotypes.

Genotypes	Incidence index (%)	Severity index (%)	ABCPE ^a	Developmental rate (r)
UTP-1	0.0 c*	0.0 c	0.0 c	0.0 c
UTP-2	79.8±0.5 b	45.6±0.4 b	861.8 b	0.17 b
UTP-4	99.4±0.3 a	50±0.3 a	1039.8 a	0.13 a
UTP-7	0.0 c	0.0 c	0.0 c	0.0 c
UTP-8	0.0 c	0.0 c	0.0 c	0.0 c

a. ABCPE = Area under the disease progression curve.

Table 4. Total yield and percentage of losses caused by antracnose on the blackberry evaluated genotypes.

Genotype	g/plant	Total yield ^a	kg/ha ^b
UTP-1 (R) ^c	5755 ± 0.3 b*	14,387 b	
UTP-2 (S)	3936 ± 0.8 c	9841 c	
UTP-4 (S)	3170 ± 0.3 c	7925 c	
UTP-7 (R)	7049 ± 0.8 a	17,624 a	
UTP-8 (R)	8400 ± 0.5 a	21,001 a	

a. = Accumulated yields during the 40 weeks of evaluation. b. = Estimated yield using a sowing density of 2500 plants/ha. c = R: Resistant genotypes S: Susceptible genotypes.

* Values on the same column followed by different letters are statistically different ($P < 0.05$), according to Tukey's test.

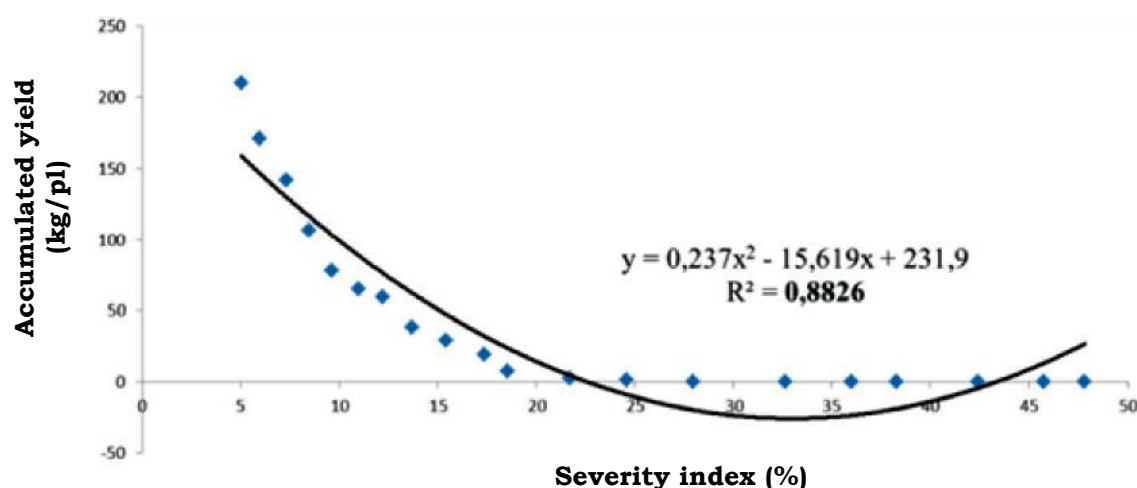


Figure 4. Correlation between the yield average and the severity index of antracnose in the susceptible genotypes (UTP-4 and UTP-2).

cate that the percentage of losses due to antracnose in blackberry could be higher than a 50%. Denoyes and Baudry (1991) found higher than 80% losses in crops of the *Rubus* genus that were susceptible to the disease and without chemical treatment, this shows the negative impact of *C. gloeosporioides*, which is catalogued as the second most important pathogen in blackberry crop after *Botrytis cinerea*

The results show that the yield potential of a susceptible genotype is totally dependent of the severity index of the disease, being null the yield when its severity is higher than 20%. With these results, disease management practices should be oriented according to the severity level in the crop, which means, preventive management should be initiated when the severity is lower than 5%, curative with severity lower or equal to 10% and, eradication methods are needed when severity is higher or equal to 20%.

Conclusions

- Antracnose symptoms in blackberry were observed 42 days after plant inoculation, when the plants were 13 months old. The symptoms were variable, from small injuries to

longitudinal CRACKS in the stem and loss of highly sporulated thorns.

- The genotypes UTP-2 and UTP-4 (control) were very susceptible to antracnose, with incidence of 79.8 and 99.4%, and severities of 45.6 and 50%, respectively. The genotype UTP-2 showed a higher rate of disease development ($r = 0.17$) in respect to the genotype UTP-4 ($r = 0.13$). The genotypes UTP-1, UTP-7 and UTP-8 did not presented disease symptoms.
- It was confirmed that weather factors such as temperature and rainfall are conditions for the expression of the disease in the field on susceptible genotypes, this is because the variability in temperature between 18.5 and 20.5 °C and an accumulated rainfall that equals or is higher than 25 mm per week are a favorable condition for the pathogen development.
- The blackberry genotype with higher yield was UTP-8 with 21 t/ha/year, followed by UTP-7 and UTP-1 with 17.6 and 14.3 t/ha/year, respectively. It was found that the severity of the disease affects directly the plant production, therefore, severities above 20% can

cause till 93% of reduction in production.

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