Characterization of Phytopathogenic Fungi, Bacteria, Nematodes and Viruses in Four Commercial Varieties of Heliconia (*Heliconia* sp.)

Caracterización de Hongos, Bacterias, Nemátodos y Virus Fitopatógenos en Cuatro Variedades Comerciales de Heliconia (*Heliconia* sp.)

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Abstract. *Analysis of 914 samples of roots, rhizomes, pseudostems, inflorescences and leaves of four commercial varieties of heliconia, cultivated at the municipality of Chinchiná–Caldas (Colombia), allowed to identify five genera of plant pathogenic fungi (**Rhizoctonia, Fusarium, Colletotrichum, Helminthosporium** and **Curvularia**), three genera of plant pathogenic bacteria (**Ralstonia, Pseudomonas** and **Erwinia**), two species of viruses (**Banana streak virus** (BSV, **Badnavirus**,) and **Cucumber mosaic virus** (CMV, Cucumovirus,)), and seven genera of plant parasitic nematodes (**Helicotylenchus, Tylenchus, Meloidogyne, Ditylenchus, Aphelenchoides, Pratylenchus**, and **Radopholus**). Of these, **Fusarium** sp., affecting pseudostems, **Pseudomonas** sp., affecting leaves and inflorescences, and the plant parasitic nematodes **Ditylenchus** sp., **Aphelenchoides** sp., **Pratylenchus** sp. and **Radopholus** sp., are new records in the heliconia production in Colombia. The most limiting diseases corresponded to leaf blight, caused by **Helminthosporium** sp.; the bacterioses, caused by **Pseudomonas** sp.; the spotted stems, caused by **Fusarium** sp.; and soft rot of the pseudostems, caused by **Erwinia** sp. The pathogenicity tests demonstrated that **Colletotrichum** sp. and **Phoma** sp. are not pathogenic in leaves; while **Fusarium** sp., inoculated in pseudostems, **Helminthosporium** sp. and **Pseudomonas** sp., inoculated in leaves, and **Colletotrichum** sp. and **Pseudomonas** sp., inoculated in inflorescences, had incidence values of 83.3, 86.6, 93.3, 100.0 and 100.0%, respectively.*

Key words: Heliconiaceae, diagnosis, diseases, pathogenicity.

Resumen. *El análisis de 914 muestras de raíces, rizomas, pseudotallos, inflorescencias y hojas de cuatro variedades comerciales de heliconia, cultivadas en el municipio de Chinchiná–Caldas (Colombia), permitieron identificar cinco géneros de hongos fitopatógenos (**Rhizoctonia, Fusarium, Colletotrichum, Helminthosporium** y **Curvularia**), tres géneros de bacterias fitopatógenas (**Ralstonia, Pseudomonas** y **Erwinia**), dos especies de virus (**Banana streak virus** (BSV, **Badnavirus**,) y **Cucumber mosaic virus** (CMV, Cucumovirus,)), y siete géneros de nematodos fitoparásitos (**Helicotylenchus, Tylenchus, Meloidogyne, Ditylenchus, Aphelenchoides, Pratylenchus** y **Radopholus**). De ellos, **Fusarium** sp., afectando pseudotallos, **Pseudomonas** sp., afectando hojas e inflorescencias, y los nematodos fitoparásitos **Ditylenchus** sp., **Aphelenchoides** sp., **Pratylenchus** sp. y **Radopholus** sp., son reportes nuevos en la producción de heliconias en Colombia. Las enfermedades más limitantes correspondieron al tizón foliar, causado por **Helminthosporium** sp.; la bacteriosis, causada por **Pseudomonas** sp.; el manchado de pseudotallos, causado por **Fusarium** sp.; la pudrición de calcetas, causada por **Erwinia** sp.; y el moko, causado por **Ralstonia solanacaearum**. Las pruebas de patogenicidad demostraron que **Colletotrichum** sp. y **Phoma** sp. no son patogénicos en hojas; mientras que **Fusarium** sp., inoculado en pseudotallos, **Helminthosporium** sp. y **Pseudomonas** sp., inoculados en hojas, y **Colletotrichum** sp. y **Pseudomonas** sp., inoculados en inflorescencias, presentaron valores de incidencia de 83,3, 86,6, 93,3, 100,0 y 100,0%, respectivamente.*

Palabras clave: Heliconiaceae diagnóstico, enfermedades, patogenicidad.

Taking under consideration the importance of heliconias (*Heliconia* sp.) cultivation in Colombia as an export product and an additional alternative for job creation plans, research must be carried out in order to determine the biotic factors limiting their production. The country has an important competitive advantage for tropical flowers production; in more than 40 years, Colombia has increased its income going from quite a few annual thousand dollars to more than US $1,094 million dollars exported during 2008. Currently, Colombia has become the first flower provider for the United States, the first worldwide carnation producer-exporter and the second worldwide flower exporter country after Holland, thus generating 183,000 jobs (99,000 direct jobs and 84,000 indirect jobs) in a 7,500 ha sown field dedicated to exportation cultivation, contributing 9.9% to the gross domestic product (GDP) and putting in 25% of the rural female employment in Colombia (Asocolflores, 2009).

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More than 250 species of the *Heliconia* genus have been described, of which 97 are registered in Colombia and 48 of these have been described as endemic species, placing the country as the biggest biodiversity center for this genus in the world. For this reason, Colombia has been ranked as a strong competitor in the international market of these flowers. It is estimated that in the Coffee Triangle and the Valle del Cauca there exist approximately 471 ha cultivated with heliconias and foliage, contributing 7% of the national export. In Risaralda, the place in which the greatest heliconia sown field area in the Coffee Triangle is concentrated, there are around 120 ha dedicated to this cultivation and the number of flower growers is higher than 150, which represent an annual income close to US $1,500,000 (Díaz, 2006; Asocolflores, 2009).

It is common to observe that when the sown field of cultivation becomes bigger, the presence of diseases caused by a diversity of phytopathogenic microorganisms also increases, which is known as the price of varietal popularity (Castaño, 2002). This situation is not foreign to the heliconias production in Colombia. In spite of this, most researchers on this species have focused in a variety of topics such as heliconia inventories, taxonomy, ecology, distribution and classification that have been found in the country, which has given up precise information and to research about the pathogenic agents affecting this genera species and, even more, the relation they have with climate elements such as precipitation, relative humidity, and temperature. The previous, has lead to the need to take measures of inappropriate handling which generate a series of environmental and economic consequences afterwards.

The only published work about the identification of diseases in Colombian heliconias has been developed by Villegas *et al*. (2005) and Alarcón (2007). The reports suggest the presence of fungi genera, such as *Fusarium, Pestalotia, Helminthosporium* and *Colletotrichum*; bacteria, such as *Erwinia paradisiaca* and *Ralstonia solanacearum*; phytoparasite nematodes of the *Meloidogyne, Helicotylenchus, Rotylenchus* and *Tylenchus* genera, without considering the *Radopholus* genus, which has been considered as the most aggressive genus in the plantain and banana cultivations in the world.

In Hawaii, Sewake and Uchida (no date) describe that diseases caused by *Bipolaris incurvata, Exserohilum* (=*Helminthosporium*) *rostratum, Pyriculariopsis* sp., *Mahabalella* sp., *Mycosphaerella* sp. and *Rhizoctonia solani* in *H. bihai* var. Lobster Claw One and *H. caribaea*, can become very limiting in heliconia production. Likewise, Nakati (no date) and Rabelo (2007) consider that *Exserohilum* sp. and *Bipolaris* sp. are the most destructive foliar pathogens of heliconia cultivation in that state.

In Venezuela, Madríz *et al*. (1991) report that *Colletotrichum musae* limits heliconias commercialization, because it produces brown with white center spots which demarcate the main leaves vein in *Heliconia caribaea*, but also affects inflorescence in which, in the initial disease stages, produces irregular and sunken brown-black spots in the bracts, and in the disease advanced stage causes widespread dry decay in the whole inflorescence.

In this research, some of the phytopathogens affecting the production in four commercial varieties of heliconias in the municipality of Chinchiná-Caldas (Colombia) were identified, as a contribution for the appropriate implementation of the cultivation management strategies. The study consisted of the identification, following Koch's postulates, of pathogens affecting the production of four heliconia commercial varieties (*H. caribaea* Lam. var. Kawachi, *H. caribaea* Lam. var. Salmon, *H. lobster* (L.) var. Orange Lobster and *H. ortotricha* (L.) var. Edge of Night). The species were selected because of their commercial importance in the national and international fields.

MATERIALS AND METHODS

Area of study. The research was carried out in el Rosario farm in the municipality of Chinchiná–Caldas (Colombia), at an altitude of 1,200 masl, 80% average relative humidity, an average temperature of 22 ºC, a 2,500 mm average annual precipitation, and with homogeneous soil characterized as Chinchiná unit. This region was selected because of its cultivated area (4.2 ha) in the species of interest for this research, and because it presents highly favorable conditions for the cultivation of heliconias in the region. For *H. caribaea* var. Kawachi and *H. caribaea* var. Salmon 1 ha sown was evaluated with the two species (0.5 ha per species), and for *H. ortotricha* var. Edge of the Night and *H. orange* lobster var. 0.72 and 2.51 ha were evaluated, respectively.

Recognition of diseases

Observation and description of symptoms in the field. In order to carry out and develop this activity, all possible combinations of symptoms of probable biotic origin, such as spots, necrosis, blockage, decay and abnormal growth, were described in the evaluated varieties.

Sample gathering. Between the months of March and December 2011 the collection of 914 samples was carried out, covering leaves, roots, corms, pseudostems, inflorescences and soil associated to plants which presented any characteristic symptom or any abnormality, in which the presence of phytopathogenic agents could be suspected. The samples with lesions from an apparently fungal origin were placed in plastic bags; for lesions apparently caused by bacteria and viruses, humid paper was used; and for the phytoparasite nematodes, soil and root samples were taken. The samples were processed in the Phytopathology Laboratory at Universidad de Caldas.

Isolation and identification of phytopathogenic agents.

Fungi: moist chambers, scrapes, cuts and dissections were prepared in order to observe the fungi characteristic structures; later, samples from the chambers were taken out, and based on French and Herbert's methodology (1980), superficial scrapes were performed in the affected areas using a scalpel, or the mycelium was removed using a sterilized dissection needle. The fungal material was observed in a LW Scientific® Revelation III light microscope, with a 40X lens, previous staining with lactophenol (20 g crystalline phenol, 20 mL lactic acid, 20 mL glycerin, 20 mL distilled water and cotton blue at 5% in water) (Castaño y del Rio, 1994). Another part of the scrape, was planted in Petri dishes with PDA (potato, dextrose and agar 39 g L^{-1} water) and then they were incubated at 25 ºC in an incubator (Precision Scientific®, Standard model 815) with the purpose of obtaining abundant sporulation in order to subsequently carry out the pathogenicity testing. In the symptoms reproduction, between four and five healthy experimental units per pathogen (inflorescences or leaves) were used, which were inoculated using a DeVilbiss® No. 15 sprinkler or a 5 mL syringe, with spores suspension adjusted to a 1.5 x 10^5 spores mL^{-1} of sterile distilled water (SDW), and using three repetitions for each inoculated pathogen. The experimental units were kept in field and laboratory conditions until the specific symptoms of each disease developed. Then, these pathogens were re-isolated again to verify the diagnosis and accomplish this way Robert Köch's postulates. The identification was based on specialized fungi taxonomic keys (Hanlin, 1990; Hanlin, 1998; Barnett and Hunter, 1998; Castaño and Salazar, 1998). All the pathogenic isolations were stored at 4 ºC in sterile bottles containing SDW, following the methodology proposed by Qiangqiang *et al*. (1998). The *Rhizoctonia* sp. and *Curvularia* sp. fungi had been subjected to pathogenicity tests by Villegas *et al*. (2005), and because of that they were not used to apply Köch's postulates in this study.

Bacteria: tests were carried out in different means of cultivation in order to observe cultural, morphologic and biochemical characteristics of bacterial cultures following the Schaad (1988) schema. For the cultural characteristics, the growth on Cetrimide agar, MacConkey agar, and semi-selective South Africa agar (SMSA) were observed; to identify the morphologic characteristics, Gram staining and Gram reconfirmation with KOH at 3% was performed; and the shape, was identified through an observation under the light microscope with a 100X objective lens with immersion oil. Finally, to identify the biochemical characteristics, the presence of oxidase, catalase, the change of color in triple sugar-iron (TSI) agar, the growth in the Hugh-Leifson medium (OF), the indol production, the mobility and the presence of sulfide acid in the SIM media, the urease production in urea agar and the starch hydrolysis, were analyzed. Additionally, DAS-ELISA testing was carried out to detect the *Ralstonia solanacearum* species. As in the methodology used for fungi diagnosis, with pure bacterial colonies isolated and cultivated in nutritive agar, a bacterial suspension adjusted to 9.0 x 10^8 UFC mL^{-1} of ADE was inoculated making wounds with an handmade inoculating device which was made from two fine sheet blades fixed on an 8 x 5 cm rectangular piece of wood. Inoculation was carried out submerging the inoculating device in the bacterial suspension, in order to subsequently cause wounds on healthy leaves or inflorescences in order to reproduce the disease symptoms. All the pathogenic isolations were stored at 4 ºC in a BHI (Brain - Heart-Infusion) medium with the purpose of carrying out their subsequent molecular characterization. *Ralstonia solanacearum* and *Erwinia* sp. microorganisms had been submitted to pathogenicity tests in the research work carried out by Villegas *et al*. (2005), reason why they are not included in this work.

Nematodes: in each sampling date, three repetitions per sample were carried out in each variety. Each sample consisted of roots, 30 g, and soil, 30 g, which contained each between eight and ten sub-samples, depending on the cultivated area. Each sample (soil and roots) was taken 25 cm around each plant and to a depth of 30 cm (Araya and Chaves, 1997). Phytonematodes extractions and quantifications were carried out using the liquidization, centrifugation and sugar flotation method (Araya, 1995; Guzmán and Castaño, 1997). This way, the number of nematodes per milliliter was obtained and the total population per analyzed sample was calculated. The specimens mounting were done in slide plates for their observation under the optical microscope. The results were expressed in number of nematodes per 100 g of soil and 100 g of roots. The identifications were made following Thorne (1961) and Mai *et al*. (1996) taxonomic keys.

Viruses: the BSV y CMV viruses were identified using the ELISA (DAS-ELISA and TAS-ELISA) commercial serological tests and following the instructions given by the manufacturer (Agdia®, United States). In all cases, the parameter of classification for positive tests was assumed when the absorbance value was twice larger than the media of negative controls.

Variables to be evaluated. The incidence (%) of all diseases found in the production of four varieties of heliconias, was determined according to observed symptomatology in the field in each affected organ (rhizome, pseudostems, pedicel, inflorescence and leave). The incidence was determined using the following formula:

$$Incidence\,(\%) = \frac{No.\ of\ \ affected\ \ plants}{No.\ of\ \ evaluated\ \ plants}\ x\ 100\%$$

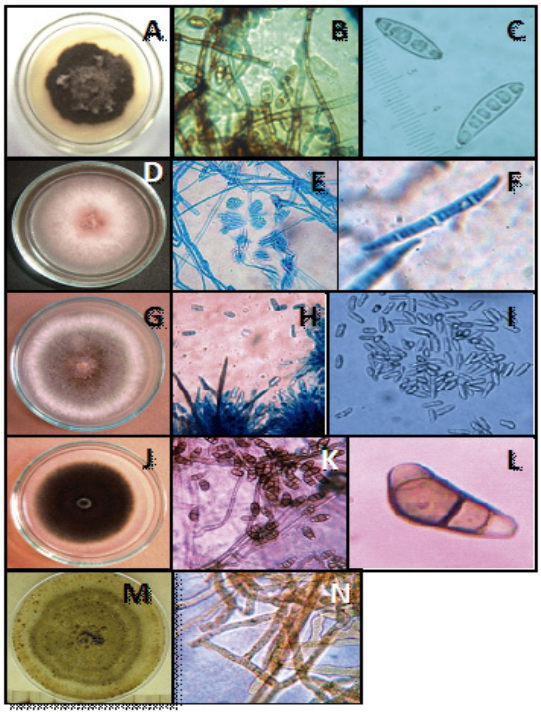

Figure 1. Genera of phytopathogenic fungi identified in the four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia). A: Mycelial growth of *Helminthosporium* sp. in PDA 15 days after sowing. B: *Helminthosporium* sp. dark brown pigmented mycelium. C: *Helminthosporium* sp. multi-cellular conidia at 40X magnification. D: *Fusarium* sp. mycelial growth in PDA 8 days after sowing. E: *Fusarium* sp. micro-conidia at 40X magnification. F: *Fusarium* sp. macro-conidia at a 100X magnification. G: *Colletotrichum* sp. growth in PDA 8 days after sowing. H: Presence of mushrooms. I: *Colletotrichum* sp. conidia at 40X magnification. J: *Curvularia* sp. growth in PDA 8 days after sowing. K and L: Four cell, dark brown with three transverse septa *Curvularia* sp. conidium. M: *Rhizoctonia* sp. mycelial growth and sclerotia production in PDA 20 days after sowing. N: *Rhizoctonia* sp. hyfas ramification in right angle.

Statistical analysis. The obtained results in the pathogenicity tests were submitted to variance analysis using the Stat graphics Plus 5.1 program, and the averages of each evaluation were analyzed using Duncan's Multiple Range Contrast test with a 95% level of confidence.

RESULTS AND DISCUSSION

Analysis of 914 samples including roots, rhizomes, pseudostems, inflorescences and leaves, belonging to the four heliconia varieties studied allowed the identification of five genera of phytopathogenic fungi (*Rhizoctonia, Fusarium, Colletotrichum, Helminthosporium* and *Curvularia*) Figure 1; three genera of phytopathogenic bacteria (*Ralstonia, Pseudomonas* and *Erwinia*) Table 1; two species of viruses (Banana Streak Virus (BSV), *Badnavirus* and Cucumber Mosaic Virus (CMV), *Cucumovirus*) Tables 2 and 3; and seven phytoparasite nematodes genera (*Helicotylenchus, Tylenchus, Meloidogyne, Ditylenchus, Aphelenchoides, Pratylenchus* and *Radopholus*) Figure 2. These results coincide with Villegas *et al*. (2005) and Alarcón (2007) results, but they include six new pathogen records affecting heliconias production in Colombia which are: *Fusarium* sp., affecting pseudostems; *Pseudomonas* sp., affecting leaves and inflorescences; and nematodes of the genera *Ditylenchus, Aphelenchoides, Pratylenchus*, and *Radopholus*.

Table 1. Consolidate of cultural, morphologic, biochemical, and serological characterization results performed on 24 bacterial isolations obtained from four varieties of heliconia cultivated in the municipality of Chinchiná–Caldas (Colombia).

Cultural characteristics			
Cetrimide agar growth	Negative	Creamy colonies	Mucous and creamy colonies
Mac Conkey agar growth	Pink mucous colonies (positive lactose)	Transparent mucous colonies (negative lactose)	Transparent mucous colonies (negative lactose)
YDC agar growth	Whitish mucous colonies	Creamy-colored mucous colonies	Creamy-colored mucous colonies
Semi selective agar South Africa (SMSA)	Negative	Burgundy with bright whitish halo and complete edges flat colonies	Burgundy with bright whitish halo and complete edges flat colonies
Morphologic characteristics			
Gram	Negative	Negative	Negative
Gram reconfirmation with KOH at 3%	Negative	Positive	Positive
Shape	Straight bacillus	Bacillus	Bacillus
Biochemical characteristics			
Oxidase	Negative	Positive	Positive
Catalase	Positive	Positive	Positive
Triple sugar iron negative agar (TSI)	A/A (acid/acid) with presence of gas	K/K (alkaline/alkaline)	K/K (alkaline/alkaline)
OF agar growth (Hugh Leifson medium)	Fermentative metabolism	Oxidative metabolism	Oxidative metabolism
Indol	Positive	Negative	Variable
Mobility	Positive	Positive	Positive
Hydrogen sulfide	Negative	Negative	Negative
Esculin hydrolysis	Negative	Positive	Positive
Gelatin liquation	Negative	Positive	Variable
Use of citrate (Simmons citrate medium)	Positive	Positive	Positive
Urease	Negative	Positive	Positive
Potato decay	Positive	Negative	Negative
Starch hydrolysis (starch agar)	Negative	Negative	Negative
Serological characteristics			
DAS-ELISA to detect *Ralstonia solanacearum*	Negative	Negative	Positive
Genera	***Erwinia***	***Pseudomonas***	***Ralstonia***

Table 2. DAS-ELISA test results for CMV in the four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia).

Variety	Plant Nº	Plants positive to BSV	Incidence (%)	Absorbance (450 nm)	Controls		DCN*
					C+	C-	
Salmon	1	-	0	0.079	0.657	0.077	0.154
	2	-		0.075	0.657	0.077	0.154
	3	-		0.070	0.657	0.077	0.154
	4	-		0.071	0.657	0.077	0.154
	5	-		0.080	0.657	0.077	0.154
Edge	1	-	0	0.070	0.657	0.077	0.154
	2	-		0.074	0.657	0.077	0.154
	3	-		0.072	0.657	0.077	0.154
	4	-		0.071	0.657	0.077	0.154
	5	-		0.081	0.657	0.077	0.154
	6	-		0.077	0.657	0.077	0.154
Orange	1	x	60	0.871	0.657	0.077	**0.154**
	2	x		0.842	0.657	0.077	**0.154**
	3	-		0.073	0.657	0.077	0.154
	4	-		0.073	0.657	0.077	0.154
	5	x		0.864	0.657	0.077	**0.154**
Kawachi	1	x	40	0.243	1.189	0.114	**0.228**
	2	-		0.093	0.263	0.102	0.204
	3	-		0.104	0.263	0.102	0.204
	4	-		0.128	0.263	0.102	0.204
	5	x		0.564	0.263	0.102	**0.204**

Table 3. DAS-ELISA test results for BSV in the four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia).

Variety	Plant Nº	Plants positive to BSV	Incidence (%)	Absorbance (450 nm)	Controls		DCN*
					C+	C-	
Salmon	1	-	0	0.078	0.152	0.123	0.246
	2	-		0.112	0.152	0.123	0.246
	3	-		0.110	0.152	0.123	0.246
	4	-		0.079	0.152	0.123	0.246
	5	-		0.102	0.152	0.123	0.246
Edge	1	-	0	0.069	0.206	0.096	0.191
	2	-		0.073	0.206	0.096	0.191
	3	-		0.071	0.206	0.096	0.191
	4	-		0.075	0.206	0.096	0.191
	5	-		0.071	0.206	0.096	0.191
	6	-		0.077	0.206	0.096	0.191
Orange	1	x	60	0.451	0.228	0.157	**0.314**
	2	-		0.219	0.918	0.165	0.330
	3	-		0.125	0.918	0.165	0.330
	4	x		0.437	0.228	0.157	**0.314**
	5	-		0.141	0.918	0.165	0.330
Kawachi	1	-	40	0.192	0.152	0.123	0.246
	2	-		0.161	0.152	0.123	0.246
	3	x		0.415	0.228	0.157	**0.314**
	4	-		0.178	0.228	0.157	0.314
	5	-		0.192	0.152	0.123	0.246

Each absorbance consolidated value represents the average of 3 repetitions
*Denotes double negative control.

Table 4. Incidence (%) of identified pathogens affecting rhizomes, pseudostems, pedicels, inflorescence and leaves in four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia).

Variety	Rhizomes	NDS[a]/ NDE[b]	I (%)[c]	Pseudostem[d]	NDS/ NDE	I (%)	Pedicels[e]	NDS/ NDE	I (%)	Inflorescence	NDS/ NDE	I (%)	Leaves	NDS/ NDE	I (%)
Salmon	*Ralstonia solanacearum*	6/10	60.0	*Erwinia* sp.	13/15	86.7	*Fusarium* sp.	13/15	86.7	*Fusarium* sp.	9/15	60.0	*Helminthosporium* sp.	12/15	80.0
	Fusarium sp.	7/15	46.7	*Fusarium* sp.	4/15	26.7				*Pseudomonas* sp.	9/15	60.0	*Pseudomonas* sp.	7/15	46.7
	Erwinia sp.	1/8	12.5							*Colletotrichum* sp.	2/15	13.3	*Curvularia* sp.	3/15	20.0
	Rhizoctonia sp.	1/10	10.0							*Pseudomonas* sp.	3/15	20.0	CMV	4/15	40.0
Kawachi	*Rhizoctonia* sp.	2/15	13.3	*Erwinia* sp.	14/15	93.3	*Fusarium* sp.	1/19	5.0				*Helminthosporium* sp.	5/15	33.3
	Fusarium sp.	1/15	6.7										*Pseudomonas* sp.	3/15	20.0
													BSV	3/15	20.0
Orange	*Erwinia* sp.	2/15	13.3	*Erwinia* sp.	9/15	60.0				*Pseudomonas* sp.	1/15	6.7	*Pseudomonas* sp.	15/15	100.0
	Fusarium sp.	1/15	6.7	*Fusarium* sp.	1/15	6.7	*Fusarium* sp.	5/15	33.3				CMV	9/15	60.0
													BSV	4/15	40.0
													Helminthosporium sp.	1/15	6.7
Edge of the Night	*Ralstonia solanacearum*	15/10	66.7	*Erwinia* sp.	15/15	100.0	*Fusarium* sp.	1/15	100.0	*Fusarium* sp.	2/15	13.3	*Helminthosporium* sp.	14/15	93.3
	Fusarium sp.	15/5	33.3	*Fusarium* sp	3/15	20.0				*Pseudomonas* sp.	1/15	6.7	*Curvularia* sp.	2/15	13.3
	Erwinia sp.	1/15	6.7							*Colletotrichum* sp.	1/15	6.7	*Pseudomonas* sp.	1/15	6.7

[a] Number of plant with symptoms; [b] Number of evaluated plants; [c] Incidence; [d] Pseudostem: A false stem composed of concentric rolled or folded blades and sheaths that surround the growing point in Musaceous; [e] Pedicel: pseudostem supporting inflorescence.

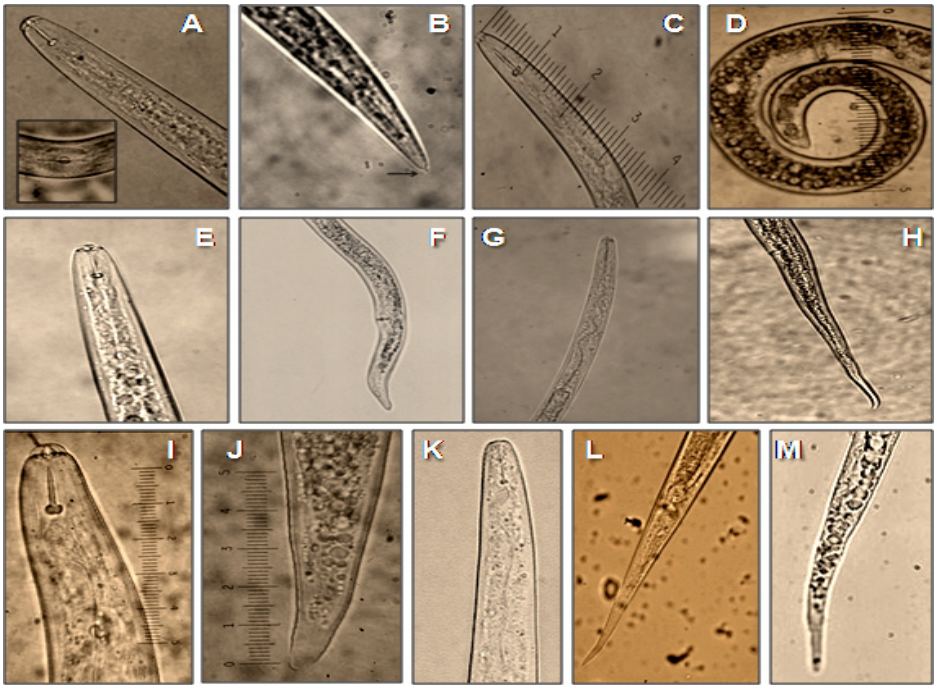

Figure 2. Phytoparasite nematode genera identified in soil and roots of four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia). A and B: Head and tail areas of a female *Aphelenchoides*, respectively; the box indicates a detail of the esophagus. C and D: Head and tail areas of a female *Helicotylenchus*, respectively. E and F: Head and tail areas of a female *Pratylenchus*, respectively. G and H: Head and tail areas of a female *Tylenchus*, respectively. I and J: Head and tail areas of a female *Radopholus*, respectively. K and L: Head and tail areas of a male *Ditylenchus*, respectively. M: Head and tail areas of a young (Y2) *Meloidogyne*.

Madríz *et al*. (1991), mention that species *H. caribaea*, *H. latispatha* and *H. psittacorum* are very susceptible to phytopathogenic fungi attack. In Venezuela, these authors report the attack of *Phyllostica musae, Glomerella cingulata, Alternaria alternata, Gloeosporium musarum, Colletotrichum musae, Guignardia musae, Curvalaria* sp., *Fusarium oxysporum, Mycosphaerella musicola, Drechslera musae-sapientum* and *Pestalotiopsis* sp. to seven species of heliconia (*H. caribaea, H. latispatha, H. psittacorum, H mariae, H. platystachys H. revoluta* and *H. rostrata*).

A record of diseases associated to heliconias production in Hawaii, developed by the Vegetal Pathology Department at University of Hawaii and the United States Agricultural Diagnostic Service Center, indicated that pathogens isolated from roots and rhizomes, such as *Calonectria spathiphylli, Phytophthora nicotianae, Pythium* sp., *Rhizoctonia solani, Ralstonia solanacearum, Radopholus similis, Meloidogyne* sp., *Pratylenchus* sp., *Rotylenchus reniformis*, and *Helicotylenchus* sp., are important for decreasing heliconias production, followed by foliar diseases which can become very destructive and are caused by *Bipolaris incurvata, Exserohilum (=Helminthosporium) rostratum, Pyriculariopsis* sp., *Mahabalella* sp. and *Mycosphaerella* sp. (Sewake and Uchida, no date).

The most frequent diseases found in this investigation (Table 4; Figure 3) corresponded to:

1) Leaf blight caused by *Helminthosporium* sp. presenting an incidence of 93.3% (Table 3) in the Edge of the Night variety whose symptoms corresponded to irregular oval brown spots surrounded by an approximately 1.5 mm in diameter chlorotic halo which can coalesce causing the drying of the whole foliar plate (Figure 3B). These results coincide with Alarcón (2010), who reported identical symptoms in *Heliconia ortotricha* var. Red and *Heliconia rostrata*. A record of diseases associated with heliconia production in Hawaii, carried out by the Plant Pathology Department at University

of Hawaii and the United States Agricultural Diagnostic Service Center, indicated that foliar diseases caused by *Bipolaris incurvata, Exserohilum (=Helminthosporium) rostratum, Pyriculariopsis* sp., *Mahabalella* sp. and *Mycosphaerella* sp. may become very limiting in heliconias production (Sewake and Uchida, no date). Similarly, Nakati (no date) and Rabelo (2007), in Brazil, consider that *Exserohilum* sp. and *Bipolaris* sp. are the most destructive foliar pathogens in heliconia cultivations in that country.

2) Bacteriosis in inflorescence and leaves caused by *Pseudomonas* sp., registering a 60% incidence in inflorescence of the Salmon variety (Table 3) and a 100% incidence in leaves in the Orange variety. Bacteria producing irregular watery spots which coalesce in order to destroy the whole foliar plate forming a big spot which advances from the foliar limb to the main leaf vein (Figure 3C). It is common to observe the presence of concentric rings in the lesions which usually confuse the evaluator with the presence of *Cordona* sp. or *Alternaria* sp. In the inflorescences, the pathogen produces brown watery lesions which can necrose the whole tissue. Usually the disease appears more frequently in the edges of inflorescence and advances to become a huge necrotic spot (Figure 3D).

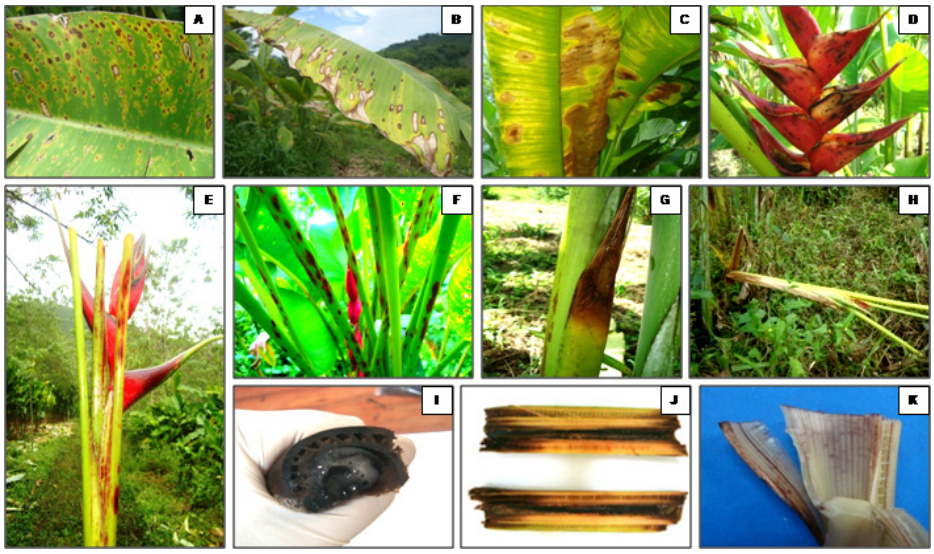

Figure 3. Most frequent diseases in the production of four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia). A-B: Leaf blight (*Helminthosporium* sp.). C-D: Bacteriosis in leaf and inflorescence, respectively. E–F: Pedicel and pseudostem stain (*Fusarium* sp.), respectively. G–H: Bacterial soft rot (*Erwinia* sp.). I-K: Moko (*Ralstonia solanacearum*).

3) Pseudostems and pedicels stained caused by *Fusarium* sp., presenting incidences from 20 to 100% in the Edge of the Night variety respectively (Table 3). The pathogen produces typical reddish spots with an elongated and rhomboid shape in pseudostems of the Edge of the Night variety (Figure 3F), and in the Orange, Kawachi and Salmon varieties. The fungus produces small, elongated, dotted and reddish spots in the pseudostems. Sick pedicels, which accompany the marketable flower, discredit their quality and represent economic losses for the flower grower (Figure 3E). In Brazil, Reis (2010) reported 31 isolations of *Fusarium oxysporum* f. sp. *cubense* affecting 88% of the tropical flowers producer pieces of land, including heliconias; the *H. bihai, H. psittacorum* cv. Golden Torch, *H. psittacorum* cv. Golden Torch Adrian, *H. rostrata, H. stricta* Capri, *H. psittacorum* cv. Sassy and *H. caribaea* species were considered as resistant to vascular wilting. The moderately resistant species were *H. latispatha* and *H. wagneriana*, while *Heliconia psittacorum* cv. Alan Carle and *H. chartacea* cv. Sexy Pink were susceptible, and *H. stricta* Fire Bird was highly susceptible. These results do not coincide with those observed in this study because the *H. caribaea* cv. Salmón species was the most susceptible to the disease. Mata *et al*. (no date) mention that from the four *Fusarium oxysporum f.* sp *cubense* existing races, race 4 is the most pathogenic in banana from the Cavendish, and Heliconias group.

4) Bacterial soft rot caused by *Erwinia* sp., reaching a 100% incidence in the Edge of Night variety (Table 3), whose symptoms are characterized by the presence of watery decay (Figure 3G), with pungent, putrid smell and with presence of bacterial exudates. At an advanced stage of the disease, the pathogen can completely necrose the pseudostem allowing the plant to bend where the lesion is located (Figure 3H). According to Belalcázar and Merchán (1991) the main cause for the disease is the plant nutritional disequilibrium, especially in boron and potassium. Among the factors increasing the disease severity are drought periods followed by heavy rain. Bacterial soft rot is a disease that must be taken into account for the integrated management of heliconia diseases, since tough the disease stays during the productive cycle affecting bacterial soft rot, it is very possible that as the bacterial inoculum concentration increases the symptoms of plants with bent pseudostems become more frequent which would change the disease into a limiting one for the production of the heliconias studied.

5) Moko caused by *Ralstonia solanacearum*, which was identified affecting rhizomes in the Salmon and Edge of the Night varieties with 60 and 66.7% incidence, respectively (Table 3), is characterized because it produces the plants death, marked wilting, mature leaves tanning, loss of swelling in the leaves, delay in growth and severe chlorosis in young leaves. At the internal level, pseudostems present decay and vascular bundles necrosis (Figures 3J and K). One of the most prominent characteristics of the disease could be observed in laboratory conditions when the affected plant organs produced abundant bacterial exudation known as Moko (Figure 3I). *R. solanacearum* is a highly aggressive phytopathogenic bacterium with a worldwide distribution and a wide range of host plants including around 50 botanic families comprising cultivations, such as potatoes (*Solanum tuberosum* L.), tomatoes (*Solanum* l*ycopersicum* L.), tobacco (*Nicotiana tabacum* L.), bananas (*Musa paradisiaca* L.), heliconias (*Heliconia* sp. L.), anthuriums (*Anthurium* sp. Schott) and peanuts (*Arachis hypogaea* L.) (Denny and Hayward, 2001). Traditionally, members of *R. solanacearum* have been subdivided in five races based on their host range and five biovars depending on their metabolic capacity for the use of diverse carbon sources; race 1 (biovars 1, 3 or 4) affects a great amount of plants including potatoes, tomatoes and the solanaceae family in general; race 2 (biovars 1 or 3) affects plantains, bananas and heliconias; race 3 (biovars 2) is considered specific for potatoes and is associated with some solanaceae family plants; race 4 (biovars 4) attacks ginger; and race 5 affects blackberry plants (*Rubus glaucus* Bentham) (Hayward, 1991).

Less frequent diseases in this research (Table 4; Figure 4) corresponded to inflorescence Antracnose (*Colletotrichum* sp.) with a 13.3% and 20% incidence in the Salmon variety, respectively. In Venezuela, Madríz *et al*. (1991) reported that *Colletotrichum musae* produces brown with white center spots which demarcate the main rib in *Heliconia caribaea* leaves; but also affect inflorescences in which, in initial stage of the disease, produce irregular and sunken brown-black spots in the bracts and, in advanced stage of the disease, causes a dry decay widespread on all the inflorescence. The *Curvularia lunata* species has been reported in Brazil, causing foliar spots in *Heliconia psittacorum* (Assis *et al*., 2002; Lins and Coelho, 2004; Costa, 2007).

Corm decay (*Rhizoctonia* sp.) presented a 13.3% incidence in the Kawachi variety. *Rhizoctonia solani* Kühn is one of the most common plants pathogen in the world. Almost every cultivation can be affected by *R. solani* or other *Rhizoctonia* species; in Hawaii, *R. solani* causes decay in roots in *H. bihai* var. Lobster Claw One and *H. caribaea* (Sewake and Uchida, no date).

BSV and CMV viruses presented a 40% and 60% incidence in the Orange variety, respectively. Plants affected by the BSV virus did not manifest delay in growth or production. In the Edge of the Night and Salmon varieties, CMV virus was not detected maybe because of the severe attack of leaf blight, caused by *Helminthosporium* sp. in this variety. This virus is not limiting in heliconia production presently, but the symptoms associated to the pathology, as well as the foliar distortion and atrophy, can become production limiting factors while the disease incidence and the cultivation area increase.

BSV and CMV can act as a viral complex causing secondary veins necrosis, stripes and mosaics in the leaves. The complex was identified in a plant from the Orange variety (Tables 2 and 3). Similar results were obtained in Dominico-Hartón plantain by López (2009). In the Department of Caldas, Dominico-Hartón plantain plants infected with BSV can present reduction in the bunch size associated with a 35%

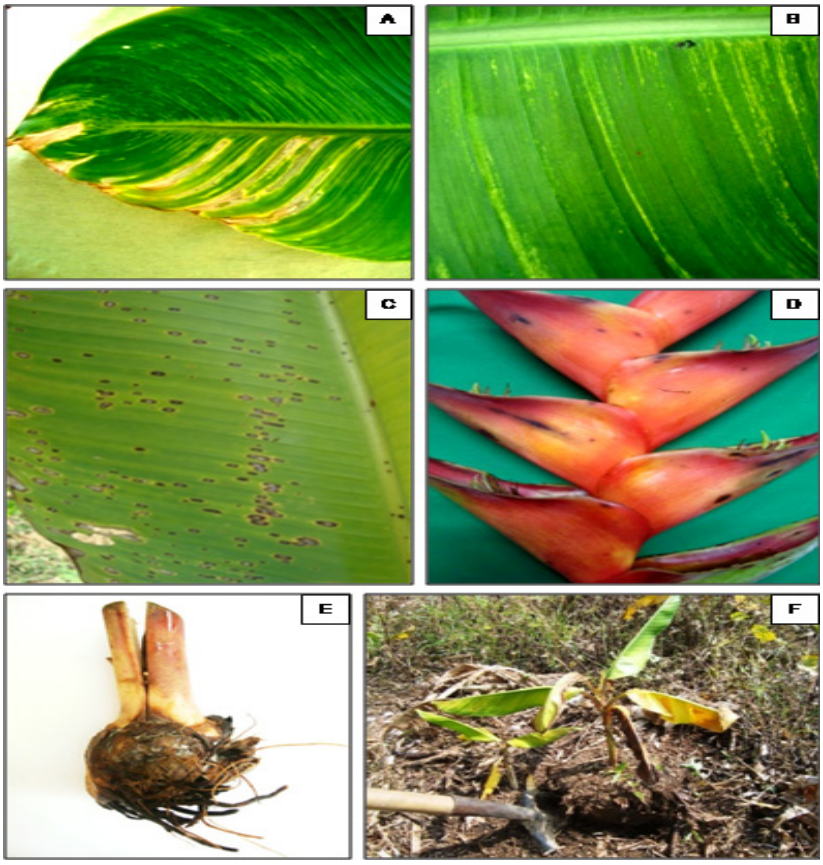

Figure 4. Diseases which can become important in the production of four heliconia varieties cultivated in the municipality of Chinchiná–Caldas (Colombia). A: Cucumber mosaic virus (CMV, *Cucumovirus*. B: Banana streak virus (BSV). C: Foliar stain (*Curvularia* sp.). D: Anthracnose of inflorescence (*Colletotrichum* sp.). E–F: Corm decay (*Rhizoctonia* sp.).

production loss; while those infected with CMV, can reduce production to a 62% (López, 2009). Belalcázar (1996) report 50% or more loss in weight for bunches produced by infected plants with CMV in Valle del Cauca.

Pathogenicity Test. Köch's postulates application allowed to clarify the registered diseases etiology in the four heliconia varieties studied (Table 5).

The application of Duncan's multiple range test (5%) allowed to conclude that *Colletotrichum* sp. and *Phoma* sp. were not pathogenic in leaves; while *Fusarium* sp., inoculated in stems, *Helminthosporium* sp. and *Pseudomonas* sp., inoculated in leaves, and *Colletotrichum* sp. and *Pseudomonas* sp., inoculated in florescence, reached 83.3, 86.6, 93.3, 100.0 and 100.0 incidence percentages, respectively (Table 5). *Pestalotia* sp., *Botrytis* sp., *Hendersonia* sp., *Nigrospora* sp., *Aspergillus* sp., *Diplodia* sp., *Rhizopus* sp., *Cladosporium* sp., *Coniotyrium* sp., *Cordana* sp., *Xanthomonas* sp., *Chaetomium* sp., *Mycosphaerella musicola, Alternaria* sp. and *Cercospora* sp., reported by Villegas *et al*. (2005), were not reported in any of the four heliconia varieties evaluated, probably because they used different heliconia varieties in that investigation.

CONCLUSIONS

The results of this investigation allowed to identify, in an heliconia commercial cultivation located in Chinchiná, Caldas, the presence of *Rhizoctonia* sp., *Fusarium* sp., *Colletotrichum* sp., *Helminthosporium* sp. and *Curvularia* sp. phytopathogenic fungi; *Ralstonia solanacearum, Pseudomonas* sp. and *Erwinia* sp. bacteria; BSV and CMV viruses,

Table 5. Results of pathogenic test of five microorganisms carried out in field conditions with different organs of the *Heliconia ortotricha* variety Edge of the Night, *Heliconia caribaea* variety Salmon, *Heliconia caribaea* variety Kawachi and *Heliconia lobster* variety orange.

Microorganism	Inoculated Organ	Inoculum Quantity	Repetition			Total	Average Incidence (%)[c]
			R_1	R_2	R_3		
Sterile distilled water	Leave/Flower	Does not apply	(0a/5b)	(0/5)	(0/5)	0/15	0 a
Colletotrichum sp.	Leave	1.5 x 10^6 conidia mL^{-1}	(0/4)	(0/4)	(0/4)	0/12	0 a
Phoma sp.	Leaves	1.95 x 10^6 conidia mL^{-1}	(0/5)	(0/5)	(0/5)	0/15	0 a
Fusarium sp.	Stems	1.7 x 10^6 conidia mL^{-1}	(4/4)	(2/4)	(4/4)	10/12	83.3 b
Helminthosporium sp.	Leaves	1.5 x 10^6 conidia mL^{-1}	(3/5)	(5/5)	(5/5)	13/15	86.6 b
Pseudomonas sp.	Leaves	9.0 x 10^8 UFC mL^{-1}	(5/5)	(5/5)	(4/5)	14/15	93.3 b
Pseudomonas sp.	Flower	9.0 x 10^8 UFC mL^{-1}	(5/5)	(5/5)	(5/5)	15/15	100.0 b
Colletotrichum sp.	Flower	1.5 x 10^6 conidia mL^{-1}	(4/4)	(4/4)	(4/4)	12/12	100.0 b

[a] Number of plants with symptoms
[b] Number of inoculated plants
[c] Different letters indicate the statistical differences according to Duncan's Multiple Range Contrast test (5%).

and *Helicotylenchus, Tylenchus, Meloidogyne, Ditylenchus, Aphelenchoides, Pratylenchus* and *Radopholus* phytoparasite nematodes. New records of pathogens, which affect heliconia production in Colombia, are included which are: *Fusarium* sp., affecting pseudostems; *Pseudomonas* sp., affecting leaves and florescence; and phytoparasite nematodes, of the *Ditylenchus, Aphelenchoides, Pratylenchus* and *Radopholus* genera.

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