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REVISTA DE CIENCIAS

Identification of Phytopathogenic Fungi in Cultivars of the Heliconiaceae Family

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Received: May 4, 2017 Accepted: Decembre 29, 2017

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Abstract

The main pathogens that affect the foliage and floral bracts of *Heliconia* cultivars in Las Heliconias National Park (Caicedonia, Colombia) were identified. Random samples were taken of foliage and floral bracts presenting symptoms of damage caused by phytopathogenic fungi. The degree of damage was described qualitatively and was correlated with the phytosanitary state of the crop. Reports of the evaluated plants indicated that 75% of the pathogens resided in the foliage and 25% resided in the floral bracts. The statistical analysis determined that the microorganisms *Aspergillus* sp., *Fusarium* sp., and *Nigrospora* sp. appeared equally in the two evaluated organs. The fungal genera found associated with plant sickness symptoms were considered to be phytopathogens. The degree of infection and development of disease varied from moderate lesions to severe infection. Reproductive fungi were present in the different plant organs studied and in organic matter residue in the soil.

Keywords: floral bracts, *Aspergillus* sp., *Fusarium* sp., *Nigrospora* sp., phytosanitary condition.

DOI: 10.25100/rc.v21i2.6701

1 Introduction

Heliconias have become in recent years an alternative crop that substitutes coffee and plantain crops in the lower thermal floors of the coffee regions of Colombia. The departments with highest production of heliconias are Antioquia, Quindío, Risaralda and northern Valle del Cauca, where production has increased considerably [(1)].

The Heliconiaceae family is composed of 250 species that are distributed in tropical regions worldwide. Colombia has the highest number of native *Heliconia* species, including 40% of the *Heliconia* species in the world [(2)]. Heliconias play an important ecological role in ecosystems, generating significant co-evolutionary relationships with other animal and plant species [(3)].

Additionally, to their contribution to the development of tropical ecosystems, *Heliconia spp*. flowers have found a place in national and international markets [(4)]. These plants are valued for their good quality, color, beauty, size and variety, contributing to the positioning of Colombia as the second world exporter of flowers, with a 10% share of the global market after the Netherlands. In 2002 *Heliconia* flowers had a value of US $665 million [(5)]. Colombia has invested greatly in exporting flowers, and is currently exporting over 50 types of flower [(6)].

The *Heliconia* market is very competitive and is related to the quality of the product, its availability and price. Its commercialization therefore demands an excellent control of disease and pests [(7)]. It is equally important to determine the phytosanitary regulations that restrict the free circulation of plant material in order to decrease the risk of introducing pests and disease that affect crops [(8)]. As the extension of areas planted with heliconias for commercial purposes has increased, the presence of diseases due to microorganisms has also increased, resulting in losses that comprise up to 30% of cultivated plants [(9)].

This study focused on characterizing phytopathogenous microorganisms found in heliconias in order to increase knowledge of the fungal diseases found in these crops, and to provide information that would allow the improvement of phytosanitary conditions that guarantee the cultivation of good quality flowers with high productivity and low production costs.

2 Materials and methods

This study was carried out at Las Heliconias National Park (Vereda Limones in Caicedonia, Valle del Cauca), located at 4°20´05´´N and 75°49´41´´W. Thirteen *Heliconia* cultivars that exhibited susceptibility to microorganisms were selected (great quantity of foliar stains and necrosis). Random samples of foliage and floral bracts presenting symptoms of deterioration caused by phytopathogenous fungi were collected. The degree of damage of the samples was described visually, and the incidence and severity of disease was evaluated. Collected samples were analyzed at the Phytopathology Laboratory of the Biology Department at the Universidad del Valle.

Fragments of healthy and diseased plant tissue were collected from the samples. Organ tissues with necrotic symptoms and brown or cream-colored stains were cut and divided into small samples that were disinfected [(10)]. Tissues were later grown in potato dextrose agar (PDA) and incubated at 28°C. When mycelial growth (spore development) was observed new isolations were obtained to purify the colonies. When sporulation of microorganisms was slow, microcultures for fungi sporulation were prepared for later characterization.

The macroscopic morphology was determined by direct observation of the colony from the front and back. Texture and shape were observed using a stereoscopic microscope Olimpus CX21 model CX21FS1 with a 10x ocular lens. To describe and identify structures a microscope Olimpus CX21model CX21FS1 with 4-10-40-100x ocular lenses was used.

The characteristics of the mycelium and reproductive structures observed were compared with those described in the morphological keys proposed by [11,12].

To carry out pathogenicity tests healthy plants from Las Heliconias National Park were used. Plants were acclimatized during 95 days in the greenhouse of the Biological Experimental station at the Universidad del Valle. For inoculation a spore suspension was prepared using sterile distilled water in a Newbauer chamber (American Optical Co Hemocytometer 0.1 mm deep). A concentration of $1x10^8$ spores/ml was used in order to obtain a number of spores that would be sufficient to reproduce necrosis and stain symptoms.

The inoculation was done by means of a puncture with a sterilized 5cc syringe, choosing the parts of the plant that were most susceptible to infection (foliage and flowers). For soft tissues the inoculum was deposited on top of the wound. The plant was later covered with a plastic bag, to avoid penetration by other microorganisms and the drying of the wound. The plants inoculated were kept at a temperature of 24-28°C and relative humidity of 65-69% to foster the development of symptoms. Evaluations were done every three days as per Koch´s postulates, to verify that the isolated microorganism was the same one observed in the field [10].

The data obtained in the field and in the laboratory were analyzed using descriptive statistics, according to frequency distribution tables that were determined using equation (1), where (F) is the frequency, (m_i) is the number of individuals in the genus, and (M_i) is the total number of individuals in all genera.

$$F=(m_i/M_t)*100 \tag{1}$$

The non-parametric chi-square test (for several proportions) was applied with a 95% confidence level in order to estimate significant differences among identified organisms in the evaluated organs (flowers and foliage).

The statistical test used is shown in equation (2), where (O_i) is the absolute observed frequency, is the expected frequency, (n) is the sample size, and (p) is the probability of obtained values according to the null hypothesis. An ANOVA and post-hoc Tukey test were carried out ($p<0.05$).

$$X^2=\sum_{i=1}^{k}\frac{(O_i-E_i)^2}{E_i} \tag{2}$$

The hypothesis tested whether significant differences ($p<0.05$) were detected in the evaluated organs (foliage and floral bracts).

3 Results and discussion

The results of the macroscopic and microscopic observations allowed the characterization of the most frequently found microorganisms in the foliage of evaluated *Heliconia* cultivars (Table. 1).

Table 1. *Phytopathogens fungi isolated from plant foliage in the Las Heliconias National Park.*

Cultivars	Phytopathogenos fungi
Heliconia bihai Orange	*Aspergillus sp.* *Fusarium sp.* *Nigrospora sp.*
Heliconia bihai Red	*Alternaria sp.* *Drechslera sp.* *Nigrospora sp.*
Heliconia bihai Gran papa	*Alternaria sp.* *Aspergillus sp.* *Drechslera sp.*
Heliconia bihai Lobster	*Nigrospora sp.*
Heliconia colinciana Red	*Aspergillus sp.* *Nigrospora sp.* *Nigrospora sp.*
Heliconia dielsiana Yellow	*Aspergillus sp.* *Papulospora sp.*
Heliconia lingulata Fan	*Drechslera sp.* *Nigrospora sp.*
Heliconia orthotricha Fucsia	*Aspergillus sp.*
Heliconia orthotrica Orange	*Nigrospora sp.*
Heliconia rostrata	*Alternaria sp.* *Aspergillus sp.* *Drechslera sp.* *Fusarium sp.* *Fusarium sp.* *Nigrospora sp.* *Trichocladium sp.*
Heliconia rostrata Erecta	*Fusarium sp.* *Fusarium sp.*
Heliconia wagneriana Splendid	*Papulospora sp.*
Heliconia wagneriana Peterson	*Alternaria sp.* *Aspergillus sp.* *Drechslera sp.* *Nigrospora sp.*

The percentage of infected sampled plants indicated that the highest proportion of infected plants showed severe infection and moderate lesions (31%), followed by plants with abundant lesions (19%), significant lesions (13%) and internal infections (6%). Disease symptoms occurred in leaves as well as floral bracts. Field evaluated samples showed that the highest degree of infection occurred in foliage (75%) and floral bracts (25%). Disease severity depends on the specific characteristics of each pathogen, on the degree of resistance of the variety, and on the environment. The interaction of these three factors determines the incidence of the disease, the degree of severity and the magnitude of performance loss [(13)].

According to [(14)] the pathogens found (Tab. 1) belong to the anamorphic Ascomycetes, which are considered of phytopathogenous interest because they cause diseases with economically important consequences.

Among sampled cultivars, *Heliconia rostrata* showed the highest susceptibility to attack by fungi, whereas *Heliconia orthotricha* cv. Fucsia was affected by a lower number of microorganisms and was affected less severely. In some of the *Heliconia* species evaluated common fungi were found on different parts of the plant or on individual organs, such as *Alternaria* sp., *Aspergillus* sp., *Dreschlera* sp., *Fusarium* sp., *Nigrospora* sp. and *Trichocladium* sp. The specificity of the pathogen to a given plant organ could be associated to environmental conditions like a temperature, Relative humidity, iradiant that foster its reproduction and development.

Phytopathogens found in flower and foliage samples were identified as *Alternaria* spp., *Aspergillus* spp., *Drechslera* spp., *Fusarium* spp., *Nigrospora* spp., *Trichocladium* spp. the most commonly found were *Fusarium* spp., *Nigrospora* spp., and *Aspergillus* spp., which are characterized as being aggressive pathogens. *Fusarium* spp. has been reported as one of the main causes of decay in species of the Heliconiaceae family [(15)] (Reis *et al*., 2008).

The organisms *Papulospora* spp., *Verticillium* spp. and *Monodictys* spp. were found in the foliage. The absence of *Cladosporium* spp., which was highly parasitic in the isolated samples of foliage, is probably due to its antagonism with *Alternaria* sp. Koch´s postulates were positive to diagnose diseases. The fungi *Alternaria sp., Aspergillus sp., Drechslera* sp., *Fusarium* sp., *Nigrospora* sp., *Trichocladium* sp., *Trichoderma* sp., *Verticillum* sp. were found to be related to foliage and floral bract diseases in the plants inoculated in the greenhouse. There was a correlation between isolated fungi and the fungi that cause symptoms in cultivated plants, which confirms that these microorganisms were the causal agents of damage.

Fusarium spp. species are widely distributed in soils and organic substrates. This characteristic allows them to colonize plants that have wounds in the bracts. The attack produces chlorosis and withering of foliage.

The presence of *Aspergillus* spp. is related to the facility of spore dispersion, which explains its elevated presence in the bracts and foliage of the cultivar. This genus is considered to have high phytopathogenous potential in *Heliconia* cultivars.

Although [16] stated that *Papulospora* sp. is found in the soil and causes disease in the roots of cultivated plants, in this study this pathogen was found to be present in the foliage. *Monodictys* sp. was also found in foliage, and it is possible that this microorganism is specific to this type of organ.

Specific richness of the genera

The Margalef index values were calculated for floral bracts and foliage with disease symptoms in the samples collected. There were no significant differences in these values, which shows that there was homogeneity in the fungi present in the different organs sampled. Table 2 presents Margalef´s Index for floral bracts and foliage with disease symptoms. Table 3 shows the presence of fungi in the evaluated organs.

Table 2. *Specific richness of the genera according to Margalef´s index*

Affected organ	Genera	Individuals	Margalef Index
Flower	10	45	2,36
Foliage	11	43	2,66

Table 3. *Presence of fungi in the evaluated organs.*

Phytopathogenous fungi	Decision	Test statistic (x^2)
Alternaria spp.	Reject Ho	X^2 6.5 - X^2 3.841
Aspergillus spp.	Accept Ho	X^2 0.625 - X^2 3.841
Drechslera spp.	Reject Ho	X^2 5.625 - X^2 3.841
Fusarium spp.	Accept Ho	X^2 1.25 - X^2 3.841
Nigrospora spp.	Accept Ho	X^2 0.625 - X^2 3.841
Trichocladium spp.	Reject Ho	X^2 12.15 - X^2 3.841
Trichocladium spp.	Reject Ho	X^2 9.25 - X^2 3.841

Results of *the statistical analysis indicated that Aspergillus sp., Fusarium sp., and Nigrospora sp. did not show significant differences compared with the other isolated microorganisms.*

4 Conclusions

According to the observed symptoms and the pathogenicity tests (Koch´s postulates) it was determined that the fungi *Alternaria* sp*, Aspergillus* sp*, Drechslera* sp., *Fusarium* sp., *Nigrospora* sp., *Trichocladium* sp., *Trichoderma* sp. and *Verticillum* sp. caused diseases in the *Heliconia* cultivars studied.

The statistical analysis showed that *Alternaria* sp., *Drechslera* sp., and *Trichocladium* sp. were significantly different in the two plant organs (foliage and flowers). These three genera also acted as saprophytes and did not show a high specificity towards the host, using any plant organ to reproduce.

Of the 13 evaluated cultivars, disease incidence was 98%. This value reflects the lack of management and control measures, which favors the establishment and propagation of pathogenic microorganisms.

Fusarium sp, Aspergillus sp., and *Nigrospora sp*. were more frequently found in the evaluated organs compared with the other isolated phytopathogens.

Heliconia rostrata was the most susceptible clone to attack by more phytopathogens fungi and *Heliconia orthotricha* cv. Fucsia had the lowest number of fungi that cause disease.

Inadequate agricultural practices in addition to environmental factors characteristic of the area generate conditions that favor the dissemination of microorganisms that cause disease.

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