Cylindrocarpon destructans VAR. *destructans* AND *Neonectria discophora* VAR. *rubi* ASSOCIATED WITH BLACK FOOT ROT ON BLACKBERRY (*Rubus glaucus* BENTH.) IN MÉRIDA, VENEZUELA

Luis Cedeño, Chrystian Carrero, Kleyra Quintero, Henry Pino and Wilmer Espinoza

SUMMARY

*In a commercial blackberry (*Rubus glaucus *Benth.) field located at El Valle, Municipality Libertador, Mérida State, Venezuela, a black foot rot disease was detected in 1999. The causal agent was identified as* Cylindrocarpon destructans *var.* destructans *(teleomorph=*Neonectria radicicola *var.* radicicola*). This pathogen attacks the roots and the crown, inducing die-back in canes. Symptoms similar to those observed in the field were reproduced on blackberry seedlings grown in soil inoculated (1.5% w/w) with barley grains colonized by the fungus. Control seedlings did not develop disease symptoms.* C. destructans *var.* destructans *was consistently isolated from the seedlings growing in contaminated soil. In January and May 2002, in areas of Miraflores (Municipality Campo Elías) and Tabay (Municipality Santos Marquina), respectively, similar damages were observed, but with the difference that the dying plants showed violet lesions on the vascular tissues in the lower parts of the canes, while the dead plants had abundant red perithecia on the neck, crown and roots. In December 2003, the same symptoms and signs were detected on dead plants at Santa Rosa (Municipality Libertador). The microorganism commonly associated with these symptoms was identified as* Neonectria discophora *var.* rubi*, sexual stage of* Cylindrocarpon ianthothele *var.* ianthothele*. This is the first documented report of* C. destructans *var.* destructans *as a cause of black foot rot disease on* R. glaucus*, and it is also the first report of* N. discophora *var.* rubi *on a* Rubus *species in South America.*

Introduction

In Venezuela blackberry (*Rubus glaucus* Benth.) production takes place in the Aragua, Barinas, Lara, Mérida, Táchira and Trujillo States. Although in the context of the national agricultural system this minor fruit-bearing species does not have an outstanding economic and social importance, in the Andean region, particularly in the Mérida State, it is popular and represents an important primary source of revenue for small and medium size producers. Their fruits are consumed fresh and processed in diverse ways as syrup, ice creams, marmalades, juice, wines and yogurt.

The main natural problems of this crop in the Venezuelan Andes are the diseases caused by the fungi *Glomerella cingulata* (Stoneman) Spaulding. & H. Schrenk, (anthracnose; Cedeño and Palacios, 1991), *Sphaerotheca macularis* (Wallr.: Fr.) Lind, (powdery mildew; Cedeño *et al.*, 1995a), *Peronospora sparsa* Berk. (downy mildew; Cedeño *et al.*, 1995b), *Coniothyrium fuckelii* Sacc. (cane blight; Cedeño and Carrero, 2000) and *Botrytis cinerea* Pers.: Fr. (gray mold), which reduce the yields, alter the quality of the fruit and diminish the productive life of the plants. Because of the high incidence of these pathogens, the current amount of blackberry in the regional market does not satisfy the demand; moreover, the fruit is of very low quality and tends to spoil quickly.

Without concerted efforts on the part of the Autonomous Agricultural Health Service (SASA) and growers to remove and destroy all infected materials in order to avoid or diminish the incidence and/or dissemination of the pathogens, the future of blackberry production in these regions is in doubt.

In May 1999, a disease of unknown cause was discovered in a commercial field of blackberry located in the village of Monterrey at El Valle, Municipality Libertador, Mérida State. Plants were affected by a die-back disease. The dying plants showed symptoms of wilt, and the dead ones had lost all their leaves. In some leaves of the dying plants necrosis was visible only at the tip and the borders, while others were completely dry and brittle; all the leaves were strongly curled toward the upper surface.

The dying floricanes had mummified fruits (dry and hard), in addition to apparently unaffected mature and immature fruits. Some mummified fruits were greenish brown, while others were dark brown to almost black. The roots and the crown of the dead plants were affected by a black rot (Figure 1). Transverse cracks were seen in the roots, and in them the cortex came off with ease, revealing light brown, dark brown and black streaks in the vascular tissues. In the internal portion of the crown of dying plants, black streaks were also seen, which contrasted with the yellowish white color of the surrounding healthy tissues. All the dead plants had a hollow central portion at the crown. Later, similar symptoms were detected in plants grown in sectors of Macho Capaz (Municipality Campo Elias) and La Azulita (Municipality Andrés Bello).

KEYWORDS / Black foot rot / Blackberry / *Cylindrocarpon* / *Neonectria* /

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Luis Cedeño. Agronomist, Universidad de Oriente, Venezuela. Master in Plant Pathology, University of Georgia, USA. Researcher, Laboratory of Phytopathology, Instituto de Investigaciones Agropecuarias (IIAP), Universidad de Los Andes (ULA), Mérida, Venezuela. Address: IIAP-ULA. P.O. Box. 77 (La Hechicera), Mérida 5101-A, Venezuela. e-mail: cedenol@ula.ve

Chrystian Carrero. Agronomist, Universidad del Zulia, Venezuela. Master in Forest Management, ULA. Researcher, Laboratory of Phytopathology, IIAP-ULA.

Kleyra Quintero. Phytotechnician, Assistant, Laboratory of Phytopathology, IIAP-ULA.

Henry Pino. Phytotechnician, Fruit Trees Section, IIAP-ULA.

Wilmer Espinoza. Agricultural Technician, General Agronomy Section, IIAP-ULA.

RESUMEN

*En una plantación comercial de mora (*Rubus glaucus *Benth.) ubicada en El Valle, Municipio Libertador, estado Mérida, Venezuela, en 1999 se detectó una enfermedad de pudrición negra del pie. El agente causal fue identificado como* Cylindrocarpon destructans *var.* destructans *(teleomorfo=* Neonectria radicicola *var.* radicicola*). Este patógeno ataca las raíces y la corona, induciendo muerte regresiva en las cañas. Síntomas similares a los observados en el campo fueron reproducidos en plántulas de mora cultivadas en suelo inoculado (1,5%, p/p) con granos de cebada colonizados por el hongo. Las plántulas control no desarrollaron síntomas de enfermedad.* C. destructans *var.* destructans *fue aislado consistentemente de las plántulas crecidas en suelo contaminado. En enero y mayo 2002, en áreas de Miraflores (Municipio Campo Elías) y Tabay (Santos Marquina), respectivamente, se observaron daños similares, pero con la diferencia que las plantas moribundas mostraron lesiones de color violeta en los tejidos vasculares de la porción basal de las cañas, mientras las plantas muertas tenían abundantes peritecios de color rojo en el cuello, la corona y las raíces. En diciembre 2003, los mismos síntomas y signos fueron detectados en plantas muertas en Santa Rosa (Municipio Libertador). El microorganismo comúnmente asociado con esos síntomas fue identificado como* Neonectria discophora *var.* rubi*, fase sexual de* Cylindrocarpon ianthothele *var.* ianthothele*. Estes es el primer reporte documentado de* C. destructans *var.* destructans *como causa de una enfermedad de pudrición negra del pie en* R. glaucus *y es también el primer reporte de* N. discophora *var.* rubi *en una especie* Rubus *en Sur América.*

RESUMO

*Em uma plantação comercial de amora (*Rubus glaucus *Benth.) situada em El Valle, Município Libertador, estado Mérida, Venezuela, em 1999 detectou-se uma enfermidade de podridão negra do pé. O agente causal foi identificado como* Cylindrocarpon destructans *var.* destructans *(teleomorfo=* Neonectria radicicola *var.* radicicola*). Este patógeno ataca as raízes e a coroa, induzindo morte regressiva na cana. Sintomas similares aos observados no campo, foram reproduzidos em plântulas de amora cultivadas no solo inoculado (1,5%, p/p) com grãos de cevada colonizados pelo fungo. As plântulas controle não desenvolveram sintomas de enfermidade.* C. destructans *var.* destructans *foi isolado consistentemente das plântulas crescidas em solo contaminado. Em janeiro e maio 2002, em áreas de Miraflores (Município Campo Elías) e Tabay (Santos Marquina), respectivamente, observaram-se danos similares, mas com a diferença que as plantas moribundas mostraram lesões de cor violeta nos tecidos vasculares da porção basal da cana, enquanto as plantas mortas tinham abundantes peritécios de cor vermelha no caule, na coroa e nas raízes. Em Dezembro 2003, os mesmos sintomas e sinais foram detectados em plantas mortas em Santa Rosa (Município Libertador). O microorganismo comumente associado com esses sintomas foi identificado como* Neonectria discophora *var.* rubi*, fase sexual de* Cylindrocarpon ianthothele *var.* ianthothele*. Este é o primeiro relatório documentado de* C. destructans *var.* destructans *como causa de uma enfermidade de podridão negra do pé em* R. glaucus *e é também o primeiro relatório de* N. discophora *var.* rubi *em uma espécie* Rubus *na América do Sul.*

In February and May 2002, on roots and crowns of plants dead by black foot rot at Miraflores and Tabay, respectively, clusters of reddish to reddish brown perithecia were commonly found (Figure 2). The anatomical and morphological features of perithecia were characteristic of members of the Order Hypocreales, Subdivision Ascomycotina (Gerlach and Nilssen, 1963; Rossman, 1983; Samuels and Brayford, 1990; Rossman *et al.*, 1999; Brayford *et al.*, 2004). In Tabay, it was common to observe a violet pigmentation on the basal portion of dying canes (Figure 3), while the roots and the crown of the dead plants showed black rot symptoms.

The present work was carried out with the purpose of establishing the identity of the microorganisms causing the described symptoms, and to evaluate their pathogenicity.

Materials and Methods

Isolation and identification of the fungal pathogens

Isolations were made from symptomatic roots and crowns collected in sectors of El Arado, La Azulita, Macho Capaz, Miraflores, Monterrey and Tabay, all located in Mérida State. After washing the material with running tap water for 1h, small pieces (ca. 2-3mm) were taken from the interface of healthy and diseased tissues; these immediately were surface sterilized with 0.5% sodium hypochlorite (NaOCl) for 3min, rinsed three times in sterile distilled water (SDW), dried on sterile absorbent paper and then plated onto Petri dishes containing water agar acidified (pH 4.5) with lactic acid (AWA). The dishes were incubated at 25 ±1°C in the dark and, later, the emergent colonies were transferred to test tubes containing slants of potato-dextrose agar (PDA; Difco). Additionally, perithecia that had been briefly surface disinfested with 0.5 NaOCl, were placed directly on AWA.

Following the procedure of Hansen and Smith (1932), 10 monoconidial and 10 monoascosporic cultures were produced, respectively, using one of the numerous mass isolates obtained from infected roots and crowns collected in the sectors where perithecia were not found, and from ascospores exuded by perithecia developed on PDA. The parameters evaluated in the monoconidial culture were the morphology of the colonies on PDA and the form and size of the asexual reproductive structures (microconidia, macroconidia and chlamydospores) produced *in vitro*, while in the monoascosporic cultures they were the morphology of the colonies on PDA, the form and size of the asexual structures (macroconidia) developed *in vitro*, and those of the sexual structures (perithecia and ascospores) formed *in situ* and *in vitro*. In both cases, the cultures were incubated at room temperature (22ºC) and 12h of light from a lamp placed at 45cm (2 tubes of fluorescent daylight F40D Extralife, Former 40W, and 2 tubes of black light Roblan 110V, BLB 40W).

The identification was carried out comparing the information registered with those published in the specialized literature (Booth, 1966, 1967; Samuels and Brayford, 1990; Brayford *et al.*, 2004). Fifty microconidia, macroconidia or chlamydospores were measured in each monoconidial culture, while in each monoascosporic isolate 50 macroconidia, 25 perithecia, and 50 ascospores were measured.

Growth rate

The selected cultures were grown in plates containing 20ml of potato-sucrose agar (PSA; Booth, 1966), cornmeal agar (CMA; Difco) or PDA, which were inoculated with disks (6mm diam) taken from 10-days-old colonies on 1.2% water agar medium. Five plates of each substratum were used and they were incubated for 7d at 22°C in the darkness. The radial growth (mm/day) of the mycelium was calculated measuring in each colony 2 perpendicular diameters, substracting the diameter of the initial inoculum and dividing the result by two.

Figure 1. Symptoms (*in nature*) of black foot rot caused by *Cylindrocarpon destructans* var. *destructans*.

Figure 2. Perithecia of *Neonectria discophora* var. *rubi (in situ)*.

Figure 3. Violet stain on the neck of canes naturally infected by *Neonectria discophora* var. *rubi*.

Pathogenicity tests

For the inoculation tests with the monoconidial culture, blackberry seedlings 6-8cm tall were used. They were grown in black polyethylene bags containing soil sterilized for 1h with dry heat at 110°C during 3 successive days. Before inoculation the seedlines were placed in a human chamber with the purpose of preventing stress resulting from transplanting. The inoculum was produced by growing the fungus in test tubes (25x150mm) containing barley grains, prepared as follows. A layer (2-3cm) of moistened sterile cotton was placed in the bottom of each tube and barley grains were placed on top of the cotton to a height of 2cm. The tubes were sealed with cotton protected with gauze and sterilized twice, with an interval of 24h, for 20min at 121°C and 15lb·in^{-2}. Each tube was inoculated with four disks (6mm diam) of mycelium taken from a 5-days-old culture grown on PDA. Cultures were incubated at room temperature (22°C) and under 12:12h (light:dark) regime for 30d. The inoculum was applied at 1.5% (w/w) and each seedling received a 1:50 dilution in SDW. Seedlings used as control only received a suspension of barley grains not colonized by the fungus. The inoculum was covered with soil and the seedlings were transferred to the greenhouse where they remained covered with bags of transparent plastic for 3d. Starting 3d after inoculation (dai), the seedlings were examined periodically to evaluate the development of foliage symptoms. Regularly, some seedlings were examined to observe damages on the roots and on the neck.

The pathogenicity tests of the monoascosporic cultures were done in 9cm disposable Petri dishes (Unestam and Stenström, 1989) containing a substratum made of granulated vermiculite, dolomitic lime and peat (VT-M Premier Sogemix, Vegetable Transplant Growing Mix, pH 7.0), which was sterilized for 1h at 121°C and 15lb·in^{-2} during 3 consecutive days. The roots of 3½ months old seedlings were placed on the substratum and the roots carefully separated, while the foliage came out through a circular hole that included the cover and the base of the dish. Ten seedlings were inoculated by wounding and ten with no wounds, applying to each one 5ml of a monoconidial suspension (1.62 macroconidia/ml) on the root system. As a control 3 wounded seedlings and 3 unwounded seedlings were used and SDW was applied on them. The dishes were sealed with a double layer of Parafilm® perforated with a dissection needle to allow aeration, covered with aluminum foil to prevent illumination of the roots, and finally placed in vertical position during 3d under the lamp (12:12h light/dark regime). Later, the seedlings were transferred to the greenhouse, where they were watered twice per week with a nutrient solution (potassium nitrate 0.5g; Epsom salts 0.5g; di-Ammonium phosphate 0.16g; calcium nitrate 0.89g; water up to 1000ml).

All the inoculation tests were carried out twice. Isolations were made from the seedlings infected experimentally to prove the Koch postulates.

Results

Isolation and identification of fungal pathogens

Only two fungi were isolated from infected crowns and roots of *R. glaucus*, and from perithecia. Based on the morphology of the asexual reproductive structures produced *in situ* and *in vitro*, they were recognized as different species of *Cylindrocarpon* Wollenw. (Booth, 1966).

One of the fungi isolated consistently formed microconidia, macroconidia, and chlamydospores. It was obtained from all the materials coming from the different geographical locations sampled, except from Miraflores and Tabay. Based on the type, morphology and size of asexual structures produced *in vitro*, the fungus was identified as *C. destructans* var. *destructans* (Zinssm.) Scholten [=*C. radicicola* Wr.] (Booth, 1966; Seifert and Axelrood, 1998), whose teleomorph, *Neonectria radicicola* (Gerlach & Nilsson; Mantiri & Samuels [=*Nectria radicicola* Gerlach & Nilsson] (Gerlach and Nilsson, 1963; Booth, 1967; Samuels and Brayford, 1990; Mantiri *et al.*, 2001) was discovered in Sweden on rotten leaves, peduncles and bulbs of *Cyclamen persicum* L. (Gerlach and Nilsson, 1963).

On PSA, HMA and PDA media, the mycelial growth of *C. destructans* var. *destructans*, averaged 3.2, 2.6 and 2.3 mm/day, respectively. On PDA the colonies produced cottony aerial mycelium that was grayish-white at first, later becoming cream-colored to light brown. From the reverse, the cultures were reddish-brown in the central portion and beige in the borders. When the dishes were opened a distinctive odor of musty earth was detected.

The microconidiophores develop as lateral phialides or terminally on short lateral branches which also may form one or more laterals, each terminating in one or more cylindrical to awl-shaped phialides. The phialides measured 18.0-35.0 x 2.5-3.0μm. The microconidia were hyaline, cylindrical to oval and 7.2 (5.6-8.8μm) x 3.9 (3.3-4.5μm). The macroconidiophores are produced as lateral branches with elongated stipe and loosely branched apex, each branch terminating in one or more phialides. The macroconidia were hyaline, cylindrical or slightly wider at the distal end, straight or curved with rounded ends and a protuberant basal scar, 1-3 septate, although occasionally some with 4 or 5 septa were observed. The 1-septate macroconidia measured 26.1 (23.1-29.2μm) x 5.8 (5.2-6.2μm) and the 2-3 septate measured 37.4 (34.5-40.3μm) x 7.4 (6.8-8.0μm). The 5-septate macroconidia averaged 37.0 (36.0-38.0μm) x 7.0μm. The chlamydospores appeared on and inside the substratum forming terminal and intercalary chains and they were spherical to elliptic in shape, hyaline at the beginning and then with thick cellular golden brown walls, 12.9 (11.3-14.5μm) x 11.4 (10.1-12.7μm) in diameter, smooth, although deposits of substances that made them appear rough were frequently observed on the surface.

The teleomorph of *C. destructans* was not found *in situ* and was not produced *in vitro*, which suggests that it is a heterothallic species.

The second *Cylindrocarpon* fungus was isolated from roots, crowns and perithecia taken from diseased plants sampled in sectors of Miraflores and Tabay. The cultures originating from perithecia always formed perithecia on PDA. Clusters of perithecia were found on roots with black rot (Miraflores) and on the neck of canes with dieback (Tabay). Perithecia were ovoid to spherical, reddish to reddish-brown, 380-700 x 350-650μm, smooth and shining, with a slightly protruding, domed and darkened apical ostiole through which ascospore were extruded in white or buff tendrils. The perithecial wall did not show a pseudoparenchymatous structure but was composed of an intertwining network of thickened hyphae. Within the perithecia paraphyses and hyaline, cylindrical asci containing 8 ascospores in uniseriate disposition, were also observed. The ascospores were hyaline, ellipsoid to subfusoid with a simple septum, 11.6 (11.0-13.0μm) x 5.0μm, flat when young, but faintly spinulose and pale brown at maturity.

After 6 weeks of growth on PDA, all the monoascosporic cultures had produced fertile perithecia similar to those found in the field. The perithecia developed mainly in clusters that, in general, shared the same stromatic base and they showed the same transition in color from white, pale yellow, bright red, dark red, amber to finally almost black as seen in nature. The ascospores were extruded in creamy white colored masses deposited around the ostiole. The anatomical and morphological characteristics, as well as the size of the perithecia and ascospores that it contained, coincided with those described for *Neonectria discophora* (Mont.) Mantiri & Samuels [=*Nectria mammoidea* W. Phillips & Plowr. var. *rubi* (Osterw.) Weese] (Brayford *et al.*, 2004), teleomorph of *C. ianthothele* var. *ianthothele* Wollenw., which differs from *C. destructans* in not producing microconidia or chlamydospores (Booth, 1966). The identity of the species was confirmed by Gary J. Samuels (Personal Communication).

On PSA, HMA and PDA, the mycelial growth of the selected monoascosporic cultures averaged 1.3, 0.5 and 0.6mm/day, respectively. On PDA the colonies showed a distinctive violet pigmentation similar to that observed on the neck of the canes infected naturally in Tabay. The colonies were floccose and the surface mycelium was violet while the aerial mycelium was yellowish brown; the colony reverse was dark.

The monoascosporic cultures also developed abundant macroconidia on violet-colored sporodochia, but no microconidia or chlamydospores, and strands of golden to dark brown hyphae. The conidiogenous cells were cylindrical, hyaline, with a collarette and thickened ring in the apex, and arose at the tips of short, much branched conidiophores. The macroconidia were hyaline, curved, cylindrical with round ends, 0-5 septate. Almost 90% of the conidia were 3-5 septate, being the 4-septate the most abundant. The 3-, 4- and 5- septate macroconidia averaged 52.6x6.8, 64.8x7.1 and 71.9x7.3μm, respectively.

The absence of microconidia and chlamydospores, together with the morphology and size of the macroconidia and violet color of the colonies confirm the identity of this species as *C. ianthothele* var. *ianthothele* Wollenw., and *Neo. discophora* (Booth, 1966, Mantiri *et al.*, 2001, Brayford *et al.*, 2004).

Pathogenicity tests

The inoculation tests with *C. destructans* var. *destructans* resulted in the production on seedlings with the same symptoms as observed in the field. When the plastic bags were removed, all the seedlings growing in contaminated soil had leaflets showing wilt symptoms and tip necrosis. The leaflets had lost their natural green color and showed the tendency to curl toward the upper surface. The necrosis began at the tip and at the borders, at first light brown and later becoming dark brown. Lightly sunken light brown, dark brown, violet, purple to black lesions were observed on the roots. The leaflets of the youngest leaves were the first to die. Some roots were completely blackened, collapsed and had lost the cortex. Of the seedlings, 60% died at 7dai and the rest died during the following two weeks. Seedlings inoculated with the control were unaffected. *C. destructans* var. *destructans* was the only microorganism consistently isolated from the seedlings experimentally infected, which demonstrates that it is the cause of black rot on the roots and crown of blackberry, *R. glaucus*.

The pathogenicity tests with *Neo. discophora* var. *rubi* were also successful. Five days after inoculation, in 70% and 40% of the seedlings inoculated with and without wounding, respectively, the younger leaves had died and other leaves showed tip necrosis and tended to curl towards the upper surface. The foliage of the control seedlings was not affected. One week later, 70% and 60% of the seedlings with and without wounding, respectively, had severe foliage damage, while the control seedlings remained unaffected. Five and one-half weeks after inoculation, 50% of the wounded seedlings and 50% of those without wounds were dead, while the control seedlings remained healthy. The roots of the dying seedlings showed the same violet color as was observed on the neck of canes affected by die-back in Tabay. Around the neck only few roots turned violet. The affected roots presented the following sequence of colors: violet, light brown, dark brown and black. The roots of dead seedlings had collapsed and blackened, and *C. ianthothele* var. *ianthothele* was frequently isolated from the roots artificially infected.

Discussion

The fungi species that formerly were considered in the genus *Nectria* are now located in the families Nectriaceae and Bionectriaceae (Rossman *et al.*, 1999). *Nectria* and *Neonectria* are included in the Nectriaceae (Rossman *et al.*, 1999, Mantiri *et al.*, 2001). Species of *Nectria* s. str. include secondary pathogens of trees; they have red, anatomically distinctive perithecia and anamorphs in the genus *Tubercularia*. The species of *Neonectria* are easily distinguished from those of *Nectria* because of their *Cylindrocarpon* anamorphs; their perithecia are anatomically and morphologically different.

On the basis of analyses of mitochondrial ribosomal DNA sequences (Mantiri *et al.*, 2001; Brayford *et al.*, 2004), the species of *Nectria* with *Cylindrocarpon* anamorphs were placed in the genus *Neonectria*, including *Neonectria discophora* and *Neonectria radicicola*. Previously, *Nectria radicicola* had been included in the Nectria radicicola-group (Samuels and Brayford, 1990) and *Nectria* was included in the Nectria mammoidea-group (Booth, 1959, 1966; Rossman, 1983; Brayford *et al.*, 2004). Each of these groups was characterized by perithecial anatomy and by anamorphs.

Booth (1966) recognized 27 species of *Cylindrocarpon* and distributed them among four groups, each distinguished by the presence or absence of microconidia and/or chlamydospores. The species included in the first group produce abundant microconidia but do not have mycelial chlamydospores. In the second group were placed those species that do not form microconidia nor chlamydospores. Those of the third group develop microconidia and chlamydospores. Those of the fourth group have chlamydospores but do not produce microconidia. Booth (1966) concluded that the name *C. radicicola* was antedated by *C. destructans* and located it in group 3; while he placed in group 2 the anamorphs of *Neo. discophora* var. *discophora* (=*N. mammoidea*) and *Neo. discophora* var. *rubi* (=*N. mammoidea* var. *rubi*). The var. *rubi* was retained as a variety of *Neo. discophora* because it has only been found on plants of the genus *Rubus* (Brayford *et al.*, 2004); however, the tendency in var. *rubi* is to produce ascospores in culture and macroconidia that are somewhat shorter and wider than those of the var. *discophora*, and to grow slower *in vitro*. Whether var. *discophora* and var. *rubi* can be separated at the species level will require a more intense comparative study using DNA sequences.

The only species of *Cylindrocarpon* that has been associated with damage (canker and die-back) on *Rubus* plants grown in Europe (Brayford, 1991) is *C. ianthothele* var. *ianthothele* whose teleomorph, *Neo. discophora* var. *rubi*, was discovered for the first time in Switzerland on rotten roots of raspberry, *R. ideaus,* (Osterwalder, 1911). Later, this microorganism was found in association with similar damage on raspberry in Scotland (Alcock, 1925) and England (Nattrass, 1927; Pethybridge, 1927). However, pathogenicity of the fungus has not been demonstrated experimentally (Alcock, 1925; Nattrass, 1927; Pethybridge, 1927) and because of that it has been considered as a secondary pathogen of stressed plants (Brayford, 1991).

C. destructans is a cosmopolitan natural inhabitant of the soil, commonly associated with roots and residues of a wide variety of woody and herbaceous plants (Booth, 1966, 1967; Kluge, 1966; Samuels and Brayford, 1990), especially in alkaline soils and, less frequently, in soils of coniferous forests (Matturi and Stentöm, 1964). This fungus is considered to be a necrotrophic and opportunistic pathogen, because it only expresses its pathogenicity on plants subjected to stress or when conditions favor its development (Unestam *et al.*, 1989; Brayford, 1991). However, in this study, the selected monoconidial strain of the fungus infected and rotted the roots of healthy, unstressed seedlings, indicating that it is a true pathogen of *R. glaucus*.

C. destructans is very sensitive to antagonism and to competition on the roots and because of that, in order for it to be able to compete successfully, it has to invade and become dominant in the weakened roots before the arrival of saprophytes (Unestam *et al.*, 1989). In normal and biologically balanced soil, *C. destructans* does not become pathogenic (Kluge, 1966), but when the microbial community of the soil is altered by treatment with water steam or fungicides, toxins produced by the fungus might accumulate at toxic levels that favor the infection of the host plants. The severity of the attack by *C. destructans* on *Pinus sylvestris* L. is increased when the continuous use of fungicides inhibits the action of the antagonists (Unestam *et al.*, 1989). *C. destructans* is a "pioneer colonizer" of roots because it has the capacity to grow and develop quickly in atmospheres with low O_2 levels that are not favorable for other fungi (Ludeking and Relab den Haan, 2002).

The pathogenicity of *C. destructans* is related to the production of a toxin that weakens and kills the root tissues of plants affected by stress caused by transplant, root prune, anaerobiosis around the roots, shade produced by high plantation density and inappropriate use of certain pesticides (Kluge, 1966; Evans *et al.*, 1967; Petäistö, 1982; Unestam and Stenström, 1989; Unestam *et al.*, 1989; Beyer-Ericson *et al.*, 1991). The stress caused by O_2 deficiency due to an excess of water in the soil (Unestam *et al.*, 1989), or by the continuous use of fungicides (Unestam *et al.*, 1989; Beyer-Ericson *et al.*, 1991), weaken the root system facilitating the infection by *C. destructans*. Apparently, the soil reaction is not a critical factor because the fungus grows substantially at pH between 2.9 and 7.9 (Unestam *et al.*, 1989). The toxin seems to have antibiotic effects on other fungi (Kluge, 1966; Unestam *et al.*, 1989) because it inhibits the growth of saprophytes on infected roots and, also, it seems it has antibiotic activity *in vitro* against *Trichoderma viride* and other fungi (Kluge, 1966).

C. destructans possesses strains that differ widely in pathogenicity (Kluge, 1966). There is an intimate correlation between the production of toxin and the degree of virulence (Kluge, 1966; Unestam *et al.*, 1989), and between virulence and the production *in vitro* of a dark-colored pigment (Unestam *et al.*, 1989). The correlation between toxin production and pathogenicity indicates that the substance, probably of phenolic nature, plays an important role in the development of the root rot that affects the seedlings of *P. sylvestris* (Kluge, 1966). According to Ahn and Lee (2001), the virulence of the *C. destructans* that attacks ginseng (*Panax quinquefolius* L.), is controlled by a double helix viral RNA.

The pathogenicity tests evidenced that both of the isolated fungi are pathogens of *R. glaucus*, but *Neo. radicicola* was more aggressive, as it killed more seedlings in a shorter time. This difference in pathogenicity could be related to the rate of growth, since on PSA, PDA and CMA, *Neo. radicicola* grew 2.5, 3.8 and 5.2 times faster than *Neo. discophora*.

The growth rate of *Neo. discophora* was very low in all the substrata used, as compared to that of *Neo. radicicola*. The disease caused by *Neo. discophora*, thus, progresses more slowly than that induced by *Neo. radicicola* and, consequently, it requires that the conditions that facilitate and favor their occurrence converge and remain in place for longer periods. In this respect it is important to point out that the symptoms and signs of the disease caused by *Neo. discophora*, in general, have only been observed during later lapses to very humid years (Nattrass, 1927; Pethybridge, 1927) or very dry years (Brayford, 1991). In 1988 a severe attack of *Neo. discophora* occurred on the red raspberry of Scotland (Brayford, 1991), after a previous year whose severe summer compacted soil around the primocanes that damaged the tissues at the point of attachment to the crown. In Tabay, Mérida, Venezuela, a region where the fungus appeared associated with 11% of dead plants, the soil remained very humid for a long time; while in Miraflores the disease was observed after three months without rain. In December 2003, the disease was seen in adult plants that died in the Institute of Agricultural Research of *Universidad de Los Andes*, located in Santa Rosa, Mérida, after several months of abundant precipitation. It is possible that the pathogenicity of this microorganism is related to an excess of humidity after damages caused by the wind followed by water logging (Brayford, 1991) or with stress caused by long lapses of deficiency or abundance of water in the soil. It is our opinion that in the sites under study, the infection takes place through the wounds caused by pruning since they are not commonly treated for healing nor are they protected with fungicides. The slow development of the infection induced by this fungus could explain why *Neo. discophora* has always been found associated with old blackberry plants, while *Neo. radicicola* has been isolated from seedlings and from young and adult plants.

This is apparently the first report of *C. destructans* var. *destructans* as a cause of black rot on the roots and the crown in a species of *Rubus*. However, it is important to point out that perithecia of *Neo. radicicola* var. *radicicola* occur in native and disturbed vegetation in Venezuela. Samuels and Brayford (1990) reported specimens collected in 1971 in the mountains of Nirgua (Carabobo State, Dumont-VE 1525, on unknown vine), and in La Carbonera (Mérida State; Dumont-VE 2512, non identified wood). These specimens are preserved in the cryptogamic herbarium of the New York Botanical Garden. The literature related to the fungi that attack *Rubus* grown in America (Farr *et al.*, 1989; Alfieri *et al.*, 1994; Seifert and Axelrood, 1998), does not include species of *Nectria* or *Neonectria*. The present study seems to constitute the first report of *Neo. discophora* var. *rubi* on a *Rubus* species in South America.

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