Short communication

Virulence of *Colletotrichum gloeosporioides* causing strawberry anthracnose disease in Tucumán, Argentina

Virulencia de *Colletotrichum gloeosporioides* causante de la enfermedad de la antracnosis en frutilla en Tucumán, Argentina

S.M. Salazar[1,2*]; S.N. Moschen[2]; M.E. Mónaco[3]; A.P. Castagnaro[3]; J.C. Díaz Ricci[3]

[1] Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán. Av. Kirchner 1900, (4000), San Miguel de Tucumán, Tucumán, Argentina. *E-mail: salazar.sergio@inta.gob.ar

[2] Estación Experimental Agropecuaria Famaillá, Instituto Nacional de Tecnología Agropecuaria. Ruta Prov. 301 km 32, (4132), Famaillá, Tucumán. Argentina.

[3] Instituto Superior de Investigaciones Biológicas (CONICET- UNT) and Instituto de Química Biológica "Dr. Bernabé Bloj", Universidad Nacional de Tucumán. Chacabuco 461, (4000), Tucumán, Argentina.

Abstract

A fungal strain with phytopathological behaviour was isolated from strawberry leaves and fruits with symptoms of anthracnose disease in Tucumán province (north-west Argentina), and it was identified as strain L9 of *Colletotrichum gloeosporioides*. Inoculation experiments on different strawberry cultivars using the isolated strain revealed dissimilar phytopathological responses. Whereas the cultivars 'Pájaro' (accession Mendoza), 'Milsei', 'Selva' and 'Seascape' triggered a typical response compatible with strain L9, the cv. 'Pájaro' (accession Cafayate) and 'Gaviota' showed a typically incompatible response.

Keywords: Anthracnose; *Colletotrichum* spp.; *Fragaria ananassa*.

Resumen

Una cepa fúngica que mostró un comportamiento fitopatológico fue aislada de hojas y frutos de frutilla que presentaban síntomas de la enfermedad antracnosis en la provincia de Tucumán (Noroeste de Argentina), y fue caracterizada como cepa L9 de *Colletotrichum gloeosporioides*. Los experimentos de desafío llevados a cabo con diferentes cultivares de frutilla frente a la cepa aislada revelaron respuestas fitopatológicas diferentes. Mientras que el cultivar 'Pájaro' accesión Mendoza, 'Milsei', 'Selva' y Seascape' desencadenaron una respuesta compatible con la cepa L9, la respuesta fue incompatible en los cultivares 'Pájaro' (accesión Cafayate) y 'Gaviota'.

Palabras clave: Antracnosis; *Colletotrichum* spp.; *Fragaria ananassa*.

Strawberry (*Fragaria ananassa* Duch.) anthracnose, also called black spot, fruit rot, or crown rot, is one of the most important diseases affecting the crop (Freeman and Katan, 1997). The term "anthracnose", introduced by Brooks in 1931, was initially used to describe a new disease of strawberry caused by *Colletotrichum fragariae* (Brooks, 1931), but the term was later generalized and used to refer to all diseases caused by fungi that belong to the genus *Colletotrichum* (Delp and Milholland, 1980; Smith and Black, 1990). These pathogens can attack crowns, leaves (petioles and leaflets), peduncles, pedicels, fruits, flowers, buds, runners and roots (Howard *et al.*, 1992; Freeman and Katan, 1997). Three species of *Colletotrichum* are known to be etiological agents of anthracnose disease in strawberry: *C. acutatum* J.H.Simmonds, *C. fragariae* Brooks and *C. gloeosporioides* (Penz.) Penz. and Sacc. in Penz. (teleomorph *Glomerella cingulata* (Stoneman) Spauld. Schrenk) (Smith, 1986; Smith and Black, 1990; Howard *et al.*, 1992; Adaskaveg and Hartin, 1997; Nam *et al.*, 2006). *Colletotrichum dematium* (Pers.) Grove (Beraha *et al.*, 1973) and species of *Gloeosporium* were also initially included as anthracnose agents, but they were later determined to be *C. acutatum* (Howard *et al.*, 1992). Traditionally, the characterization of these fungal pathogens has been mainly based on classical microbiological methods, which consider colony color and morphology, conidium size and shape, presence or absence of setae and the existence of the teleomorph *G. ciculata*/*G. cingulata* (Smith and Black,

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1990; Gunnell and Gubler, 1992; Sutton, 1992; Denoyes and Baudy, 1995; Freeman *et al.*, 1998; Riegel *et al.*, 2010). *Colletotrichum gloeosporioides* has been reported in a large number of host species, but its occurrence in strawberry in the EU is only occasional (Garrido *et al.*, 2008). When plants and fruit samples with anthracnose symptoms collected in the crop area of northwestern Argentina were studied in detail, results revealed that the etiological agents corresponded to *C. fragariae*, *C. acutatum* and, to a much lesser extent, *C. gloeosporioides* (Mena *et al.*, 1974; Mónaco *et al.*, 2000; Ramallo *et al.*, 2000). Since tolerance or disease symptoms in a specific strawberry genotype depends not only on the particular pathogen strain, but also on plant tissue and inoculation procedure (Smith and Black, 1990; Mertely and Legard, 2004), this study aimed to perform a phytopathological evaluation with all the pathogens isolated. The trial consisted in spraying conidia on unwounded plants and analyzing disease symptoms on leaves (petioles and leaflets) at different times.

Fungal isolate L9 of *Colletotrichum gloeosporioides* was collected from symptomatic cv. 'Chandler' strawberry in Lules (Tucumán, Argentina) (Mónaco *et al.*, 2000). Isolates were characterized based on several criteria. Potato dextrose agar (PDA) was used to evaluate cultural and morphological characteristics of the isolate. Isolates were single-spore propagated to obtain pure cultures, and maintained on PDA slants at 4 ºC. The isolate L9 was selected for phytopathological characterization. This strain was also sent to CABI BioScience (International Mycology Institute, UK Centre, Egham, England) for its molecular characterization.

Cultures were obtained by transferring a 4 mm-diameter mycelium plug from a PDA culture to newly prepared PDA in a 90 mm diameter Petri plate. For all the experiments, cultures were incubated at 28 ºC for 12 days, under continuous fluorescent light to induce conidium formation (Smith and Black, 1990; Sutton, 1992; Mertely and Legard, 2004). Three replicate plates were prepared, and growth rate was determined by measuring colony diameter after twelve days of incubation. Conidial suspensions prepared in sterile distilled water were observed under an Olympus BH-2 microscope, and their shape was determined by examining 100 randomly chosen samples. The isolate L9 was classified into the following category: straight cylindrical body with both ends rounded. Aberrant and ambiguous conidial shapes represented less than 10 % of the population analyzed. Conidial size and perithecia were observed and photographed with a JEOL JSM35 CF scanning electron microscope, measuring the length and width of 100 conidia randomly chosen from the isolate. These measures were expressed as an average. In order to evaluate phytopathological responses, inoculation experiments were performed. The culture surface was scraped gently with a Pasteur pipette to remove conidia, and these were suspended in sterile distilled water. Conidial suspensions obtained were filtered through gauze to remove mycelial debris under axenic conditions. Suspensions were then diluted with sterile distilled water containing two drops of Tween 20 per liter to a final concentration of 1.5×10^6 conidia per ml, and the plants were sprayed with this preparation until run-off (Smith and Black, 1990; Mertely and Legard, 2004; MacKenzie *et al.*, 2009). The experimental design was randomized with eight plants per genotype and per experimental unit, four of which corresponded to the challenged plants, and four to the control plants. All experiments were repeated at least twice or more times under identical experimental conditions when results were not clear. Immediately after inoculation, plants were placed in a dew chamber with 100 % RH at 28-30 ºC (infection chamber), for 48 h in the dark. Then the plants were returned to the greenhouse (Smith and Black, 1990; Nam *et al.*, 2006).

Strawberry plants of the cultivars 'Pájaro', accessions Mendoza and Cafayate (García *et al.*, 2002), 'Camarosa', 'Chandler', 'Milsei', 'Sweet Charlie', 'Selva', 'Seascape' and 'Gaviota' were used in the experiments. Plants for phytopathological studies were obtained from runners of these cultivars, which were rooted in sterile substrate under axenic conditions. Plants were grown for 14 to 16 weeks and maintained in growth cabinets at 28 ºC, with 70 % RH and a light cycle of 16 hours per day. The ability of isolate L9 to infect different strawberry cultivars was tested by spraying the leaves and petioles of four plants of each cultivar with a suspension of 1.5×10^6 conidia per milliliter. Four uninoculated plants were used as controls. Plants were placed in the infection chamber for 48 h, and then transferred to a growth cabinet bench for evaluating disease symptoms and severity. After 50 days incubation, pathogens were isolated from crowns of plants that developed the disease, and they were compared with the strains for inoculation to check pathogen identity. Disease severity rate (DSR) was evaluated and scored on a scale ranging from 1 to 5, using a

disease index based on petiole symptoms (Delp and Milholland, 1980). Plants with DSR greater than 3 were considered susceptible.

Experimental data obtained from phytopathological tests were analyzed with the Statistix program (Analytical Software, 1996). LSD Fisher test was used for determining the arithmetic mean of the DSR value (significance level, 0.05) for each genotype of the inoculated plants, and the Analysis of Variance test (ANOVA) was used for evaluating data dispersion with respect to the mean value. Experiments were repeated three times to diminish the dispersion of DSR values.

The strain L9 presented white to pale beige colonies with abundant aerial mycelium, and the underside of the colony was gray to dark-olive near the center, and white at the edges of the colony (Figure 1A-B). Growth rate analysis showed that after 12 days of incubation on PDA at 28 ºC and under continuous fluorescent light, average colony diameter was 8.3 cm for the isolate L9 of *C. gloeosporioides* (standard deviation σ_{n-1} = 0.5 cm).

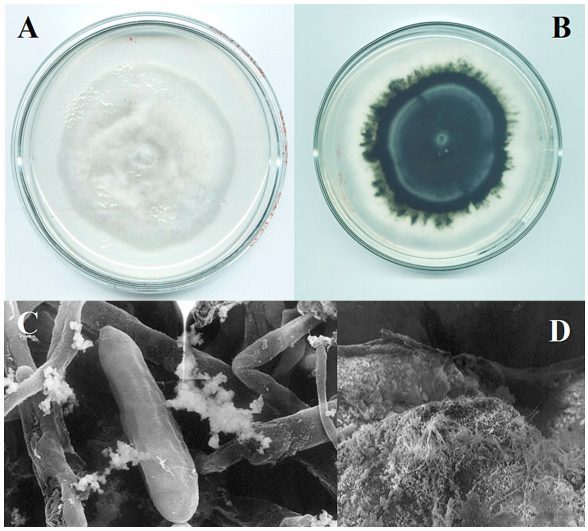

Figure 1. A. Upper (left) and **B.** lower (right) colony surfaces of isolate L9 of *Colletotrichum gloeosporioides*, **C.** SEM of conidia of isolate L9 and **D.** SEM of sexual fruiting structures (perithecia) of isolate L9. Scale bars denote 1 µm in (C) and 100 µm in (D).

Isolate L9 produced conidia with a straight cylindrical body, with one pointed end and another which was rounded. The average of conidium sizes observed was 14.5 µm long x 4.5 µm wide. The strain L9 produced perithecia in six-week-old cultures, with asci and ascospores typical of *Glomerella cingulata*, as described by Mónaco *et al.* (2000) (Figure 1D) (Strain L9 authenticated by Commonwealth Mycological Institute (CABI) – IMI number 386396).

The phytopathological evaluation of the nine strawberry cultivars inoculated with strain L9 showed differences among cultivars. Plants of cv. 'Pájaro' (accession Mendoza) showed lesions and strangulation of petioles. DSR values of cvs. 'Pájaro' (accession Mendoza), 'Milsei', 'Selva' and 'Seascape' displayed a strong compatible interaction, with a DSR ≈ 5 (Table 1). In the case of cv. 'Pájaro' (accession Mendoza), the maximum DSR value (5.0) was obtained 10 days after inoculation, whereas cv. 'Pájaro' (accession Cafayate) plants showed minor lesions or no disease symptoms 50 days after inoculation.

When 'Camarosa', 'Chandler', 'Sweet Charlie' and 'Gaviota' plants were infected, they showed no significant differences regarding disease symptoms and evolution (Table 1). Only few lesions and strangulation of petioles were observed. It is noteworthy that cv. 'Pájaro' (accession Mendoza), 'Milsei', 'Selva' and 'Seascape' reached the maximum DSR value 9-21 days after infection with strain L9, whereas cv. 'Pájaro' (accession Cafayate) and 'Gaviota' showed an incompatible interaction with minor lesions or no disease symptoms (even many months after the inoculation) and petiole strangulation was not observed in these cultivars (Table 1).

Table 1. Disease severity rates (DSR) of strawberry cultivars infected with L9 *Colletotrichum gloeosporioides* strain.

Cultivar	DSR
'Pájaro' acc. Mendoza	5.00 ± 0.25 a
'Pájaro' acc. Cafayate	1.10 ± 0.06 b
'Camarosa'	1.85 ± 0.11 b
'Chandler'	2.20 ± 0.15 b
'Milsei'	4.95 ± 0.45 a
'Sweet Charlie'	1.90 ± 0.15 b
'Selva'	4.75 ± 0.33 a
'Seascape'	4.74 ± 0.24 a
'Gaviota'	1.35 ± 0.07 b

Different letters indicate significant differences (LSD Fisher test, $P < 0.05$).

Results of cultural and morphological studies also showed that isolate L9 presents traits typical of *C. gloeosporioides* (Smith and Black, 1990; Mónaco *et al.*, 2000; Garrido *et al.*, 2008; Nguyen *et al.*, 2010). Comparative phytopathological analysis showed that regardless of disease severity degree, the nine strawberry genotypes used in this study presented qualitatively similar symptoms. Our results showed that whereas cv. 'Pájaro' (accession Mendoza), 'Milsei', 'Selva' and 'Seascape' displayed the highest susceptibility toward the isolate L9, cv. 'Pájaro' (accession Cafayate) and 'Gaviota' showed a totally different behavior. These results also revealed that

each strawberry cultivar can respond differently when challenged with the same pathogen isolate. This suggests that the strawberry-*Colletotrichum* interaction has to be considered on a genotype basis, as each cultivar may respond with a different tolerance degree depending on the particular dominant pathogen present in the fungal complex at the moment of infection (Smith and Black, 1990; Denoyes Rothan *et al.*, 1999; MacKenzie *et al.*, 2009). Hence, it becomes important to undertake thorough assessment of the pathogen populations present in an agroecological system, and to perform a correct phytophathological evaluation of these genotypes with the strawberry cultivars used in that particular system.

Taking into account these results, we may conclude that in the case of strawberry, it is more important to assess the fraction of virulent pathogen genotypes present in a specific crop area (in relation to the principal cultivar grown there) than the composition of the population of *Colletotrichum* species present in that area. Therefore, the main pathogen population exchange factor operating in the crop area after some time would be the particular cultivar used by farmers.

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