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Comparative Pathogenicity of *Colletotrichum* spp. Against Different Varieties of Strawberry Plants *(Fragaria ananassa)* Widely Grown in Morocco

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The evolution of anthracnose symptoms on the aerial part (leaves, stems and strawberries) of three varieties Fortuna, Camarosa and Festival of strawberry plants inoculated with the conidial suspensions of *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* isolates was followed. The severity index and infection coefficients increased in function of time. Seven days after inoculation they were low not exceeding 13.43% and 43.33, but they increased four weeks after inoculation, respectively, to 37.96% and 99 on strawberry plants of the Camarosa variety, 54.44% and 105 on those of Fortuna and 51.12% and 85 on those of Festival. At the sixth week, the severity index and infection coefficients became very high, reaching respectively 100% and 408 on Fortuna plants inoculated with *C. gloeosporioides* isolate (Coll3) followed by Coll2 (89.28% – 300), Coll1 (86.66% – 378) and Coll4 (80.45% – 198) of *C. acutatum* species. Similarly, the isolate Coll3 caused fruit rot; the percentage of rotten strawberries was 100% on Fortuna variety, 83.33% on Festival and 70.25% on Camarosa. A positive re-isolation of the tested *Colletotrichum* isolates has been noted from leaves of strawberry varieties and negative from crowns or the roots. A significant to moderate reduction in fresh and dry weights of the aerial part and roots was noted in inoculated strawberry plants compared to the control.

Keywords: *Colletorichum* spp., strawberry plant, pathogenecity, symptoms.

The strawberry (*Fragaria × ananassa* Duchense ex Rozier) is considered the most economically important berry worldwide (Hummer and Hancock, 2009). Developed in Europe in 1766 (Darrow, 1966), *Fragaria × ananassa* Duchesne has diversified over time into several varieties and cultivars. In particular, characteristics such as productivity, disease resistance, fruit firmness, freezing quality, sugar and acid content, flower color, habit of flowering, berry taste, size, color and shape are important for selection of strawberry cultivars (Kosar et al., 2004; Voca et al., 2009; Winardiantika et al., 2015).

Strawberry (*Fragaria×ananassa* Duch.) can be cultivated in the open field as a perennial or annual crop (Galletta and Bringhurst, 1990; Rubio et al., 2014). However, high-tunnels could mitigate some phytosanitary problems by reducing the free water on the plants and fruits (Jensen and Malter, 1995; Lamont, 2009; Grijalba et al., 2015). Protected culture systems such as greenhouses and tunnels are becoming more popular for the

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protection against frost (Neri et al., 2012), extension of the harvest period and increased yields (Lieten and Baetes, 1991; Grijalba et al., 2015), fruit quality improvement (Xiao et al., 2001; Kadir et al., 2006) as well as control of several major plant diseases (Xiao et al., 2001; Evenhuis and Wanten, 2006). Indeed, in warmer regions like California, Florida and the Mediterranean region greenhouse and tunnel strawberry production is as extensive as open fields (Delp and Milholland, 1981; Howard et al., 1992; Tanji et al., 2014; Anonymous, 2015). Concerning the conventional fruit production is based on annual planting of bare-root green plants placed in two rows into beds protected by opaque plastic mulch with localized irrigation equipment, fertilizing irrigation system and covered by macrotunnels with clear mulch (López-Aranda, 2008).

In Morocco, Gharb and Loukkos are Morocco's leading strawberry producer regions with acreage of about 3500 ha (Anonymous, 2014). The most cultivated strawberry varieties in Morocco are derived from California (Atta Aly and Ezzat, 1999). In 2013, producers were interested in planting many varieties like Camarosa, Festival, Splendor, Fortuna, Lusa, Magdalena, Sabrina, San Andreas, Venicia and Ventana but the dominant role is still played by the first four varieties (Tanji et al., 2014).

Otherwise, the growth of strawberry has increased during last decades however its productivity is still low compared to other developing countries (Anonymous, 2017). There are several reasons for it; mostly yield losses can be caused both by unfavorable environment conditions and due to damage by different pathogenic organisms (Wittwer and Castilla, 1995; Xiao et al., 2001; Jiang et al., 2003). The strawberry crop can be attacked by viruses (Martin et al., 2004; Li and Yang, 2011), bacteria (Mdarhri, 2005; Anonymous, 2006; Bull et al., 2009), mites (Zahdali, 2003; Lagziri and El Amrani, 2009), nematodes (Lamindia, 2002), pests (Nicolov, 2006), weeds (Lansari, 1985), and fungi (Paulus, 1990; Maas, 1998) including soil-borne pathogens and aerial ones. Indeed fungal species cause serious pathologies afflicting host damage and a considerable reduction of the yield (Sreenivasaprasad and Talhinhas, 2005; Abdel-Sattar et al., 2008; Fang et al., 2012; Ceja-Torres et al., 2014).

Colletotrichum is among ten most important plant pathogenic fungi (Dean et al., 2012). The genus *Colletotrichum* is highly recognized as anthracnose disease agent on strawberry (De Los Santos et al., 2003). This disease represents a potential threat and a disease of great importance whose causative agent is only the fungal complex including *C. acutatum* J. H. Simmonds, *C. gloeoporioides* (Penz.) Penz. and Sacc. (teleomorph *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk) and *C. fragariae* Brooks (Smith, 1986; Smith and Black, 1990; Gunnell and Gubler, 1992; Howard et al., 1992; Denoyes and Baudry, 1995; Freeman and Katan, 1997; Legard, 2000; De Los Santos et al., 2003; Talubnak and Soytong, 2010). Symptoms associated with *Colletotrichum* infection include crown rot (Horn and Carver, 1962; Mangandi et al., 2015) fruit rot (Howard, 1972; Urena-Padilla et al., 2002), root necrosis (Freeman and Katan, 1997), black spots on leaves (Howard and Albregts, 1983), petioles and runners (Delp and Milholland, 1981).

Indeed*, Colletotrichum gloeosporioides* and *Colletotrichum acutatum* are distributed worldwide on a number of hosts such as kernels (Ogawa and English, 1991; Adaskaveg and Hartin, 1997; Förster and Adaskaveg, 1999), avocado (Freeman, 2000; Akgül et al., 2016), peach (Adaskaveg and Hartin, 1997; Zaitlin et al., 2000), blueberries (Smith et al., 1996; Schilder et al., 2001; Yoshida and Tsukiboshi, 2002), citrus (Zulfiqar et al., 1996; Timmer and Brown, 2000; Benyahia et al., 2003), mango (Fitzell, 1979; Arauz,

2000), olive tree (Martin and García-Figueres, 1999; Benyahia et al., 2003; Chattaoui et al., 2016; Msairi et al., 2017), while *Colletotrichum fragariae* has a very narrow range of hosts (Mackenzie et al., 2006). A typical anthracnose symptom on cultivated strawberry caused by *C. acutatum* and *C. gloeosporioides* was reported in Egypt (Embaby et al., 2009). In Europe, *C. acutatum* is the most prevalent species causing anthracnose, whereas *C. gloeosporioides* is found only occasionally, and *C. fragariae* has not yet been observed (Denoyes and Baudry, 1995; Garrido et al., 2008). In UK, the first incidence of anthracnose disease in strawberries caused by *C. acutatum* was attributed to the importation of infected strawberry runners from the USA (Calleja et al., 2012). These fungal species affects fruit quality and causes significant pre- and post-harvest fruit losses, especially during wet weather (Prusky and Plumbey, 1992; Prusky, 1996; Arauz, 2000; Chrys, 2006). In Belgian strawberry production fields, fruit losses caused by *Colletotrichum* can easily rise above 25%, even after repeated treatment with fungicides (Van Hemelrijck et al., 2010). An epidemic proportion in Israeli nurseries and production fields was reached by strawberry anthracnose (Freeman and Katan, 1997) in addition to severe outbreaks of fruit rot which have been developed in the United States after the apparent introduction of *Colletotrichum acutatum* in the 1980s (Smith, 1986; Howard et al., 1992).

In Morocco, the occurrence of *Colletotrichum* species was represented by *C. acutatum* and *C. gloeosporioides* encountered in two strawberry farms in the locality of Moulay Bousselham (Gharb-Loukkos, North West Morocco) during surveys carried out in 2010 (Mouden et al., 2013). They were also appeared on the aerial parts of four varieties grown at different strawberry plantations of the Gharb-Loukkos during crop season 2012–2013 (Mouden et al., 2016). On the other hand, under laboratory conditions, one of the identified *C. gloeosporioides* isolates had the capacity to cause necrosis on detached leaves of Festival and Splendor varieties (Mouden, 2015). According to Han et al. (2016), the frequencies and distribution of *Colletotrichum* species are related to differences in high temperature tolerance and pathogenicity.

Thus, performing pathogenicity tests of different isolates of *Colletotrichum* species is required before determination of isolates aggressiveness, the risk evaluation of spread and disease development. The primary objective of this work was to study the pathogenicity of *C. acutatum* and *C. gloeosporioides* on different varieties of strawberry plants widely grown in Morocco.

Materials and Methods

Fungal material

Four isolates were used; three of them represent *Colletotrichum acutatum* (Coll1, Coll2 and Coll4). They were isolated, respectively, from a strawberry of Camarosa variety, stem of Festival variety and the crown of Camarosa variety. The fourth isolate represents *Colletotrichum gloeosporioides* (Coll3) was isolated from the crown of the Camarosa variety. The culture of four isolates were grown on PSA medium (Potato: 200 g, Sucrose: 20 g, Agar-agar: 15 g, Distilled water: 1000 mL) at 24 °C in the dark for seven days.

Plant material

Bare-root strawberry transplant of the Fortuna variety, Festival and Camarosa varieties were grown in pots containing 50% black peat and 50% of Mamora soil (Physicochemical parameters: pH: 7.53; Organic matter: 0.7%; Nitrogen: 0.05%; Phosphorus (P_2O_5): 0.239%; Potassium (K_2O): 0.15 meq/100 g; Magnesium (Mg): 0.20 meq/100 g; Calcium (Ca): 7351.5 (meq/100 g), placed in a greenhouse and daily watered until to the stage of five leaves required for inoculation.

Inoculation

Conidial suspensions of *Colletotricum* isolates were prepared by flooding fully grown potato dextrose agar (PDA) 7 days old cultures with sterile distilled water and display the surface with a glass rod. The mixture was filtered through muslin cloth into a flask with sterile distilled water containing a single drop of Tween 20 and gelatin 0.05%. The final concentration of the suspension was adjusted to 10^5 conidia/mL using the hemocytometer. Approximately, 30 mL of the conidial suspension was sprayed to runoff directly to the aerial parts of the three strawberry varieties by hand-pressurized atomizer. Plants using as control were inoculated with distilled water containing 0.05% Tween 20 and gelatin 0.05%. The inoculated plants were packed for 72 hours in plastic wrap to maintain the necessary humidity and exposure to moisture leaves for growth of the pathogen and then placed in a greenhouse. The experimental protocol was designed in random blocks with three replicates for each isolate and one plant per pot.

Disease evaluation

The symptoms appearance was followed during 6 weeks and evaluated in terms of both disease severity (extent of diseased leaf area) and disease incidence (number of leaves with symptoms).

Disease severity index

From the first week to the sixth after inoculation, the diseased leaf area was estimated according to the scale of Stover modified by Gauhl et al. (1995): degree 0=no symptoms; degree 1=less than 0.5% of the leaf showing symptoms; degree 2=0.6 to 5% of the leaf with symptoms; degree 3=6 to 15% of the leaf with symptoms; degree 4=16 to 30% of the leaf with symptoms; degree 5=31 to 50% of the leaf with symptoms; degree 6=51 to 80% of the leaf with symptoms; degree 7: 81 to 100% of the leaf with symptoms.

The disease severity index (SI) was calculated according to the following formula:

$$SI\ (\%) = \frac{\Sigma nb}{(N-1)\times T} \times 100$$

n = number of leaves for each degree of the scale;
b = degree of the arbitrary scale representing the severity;
N = number of degrees used in the scale;
T = total number of leaves evaluated.

Coefficient of infection

The coefficient of infection (IC) was calculated by multiplying the incidence by the disease severity.

IC=Severity×Incidence

Severity: degree of scale.
Incidence: total number of leaves with symptoms.

Strawberry responses to Colletotrichum *isolates*

Strawberry infections were scored every week by counting the total number of fruits showing anthracnose rot among total number of strawberries produced by each strawberry plant in function of time and determining the percentage of strawberries showing anthracnose fruit rot.

Agronomic parameters

At the end of the trials, the plants were gently taken off from their culture substrate and washed under a running water to remove adhering soil particles. The aerial part of each plant was cut at the collar, the fresh weights of the aerial part and the roots of the strawberry plants were measured using a precision balance. The dry weight of the aerial part and roots were also weighed after drying in an oven at 70 °C for 24 hours.

Re-isolation

The leaves, crowns and roots of the three varieties of strawberry inoculated with the four isolates of *Colletotrichum* and the control plants were cut into small pieces (of 1 cm^2 for leaves and 1 cm in lengh for stems and roots) and then placed in alcohol at 95 °C for 2 min, rinsed with sterile distilled water several times and dried on sterile filter paper. The fragments were planted in PSA medium amended with 50 mg Streptomycin. The Petri dishes were incubated in the darkness at 22 °C. After 10 to 14 days, the colonies were identified. The re-isolation percentage (Pr%) was obtained by applying the following formula:

$$Pr=Ns\ Px\ /\ NT\times 100$$

Ns Px: number of segments containing the fungal species x.
NT: total number of segments.

Statistical analysis

The statistical treatment of the results obtained was performed according to the generalized linear model (GLM) by comparison of means, according to the LSD test at the 5% threshold. The analyses were based on the averages attributed to the effect of each isolate on strawberry plant varieties as a function of time.

Results

The examination of the aerial plant parts showed the ability of the tested *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* isolates infecting vigorously the vegetative tissues of the three inoculated varieties of strawberry plants. The post-inoculation infections caused various disorders affecting the production of strawberry plants (devel-

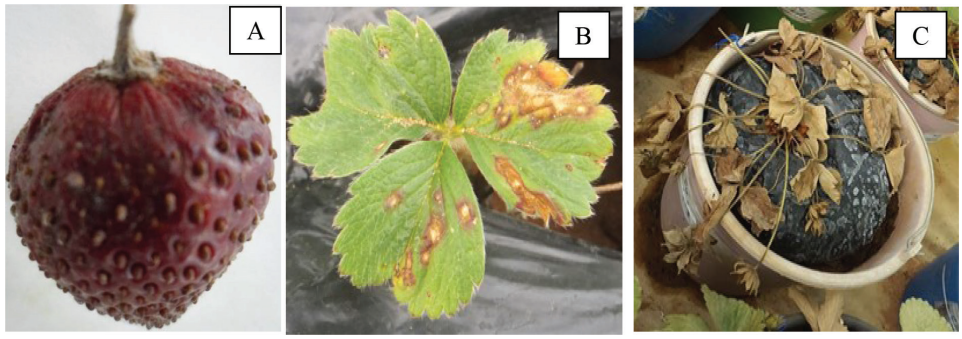

Fig. 1. Symptoms developed on a young strawberry (A), leaves (B), strawberry plant (C) after inoculation with *Colletotrichum* isolates

Table 1

Evolution of the severity index (SI%) of the disease in strawberry plants inoculated with *Colletotrichum* isolates according to time

Time (per week)	Varieties	Control	Coll1	Coll2	Coll3	Coll4
	Camarosa	0.00^{w}	3.45^{vw}	0^{w}	10.49^{rstuvw}	0^{w}
1	Fortuna	0.00^{w}	8.33^{stuvw}	7.57^{stuvw}	$13.43^{pqrstuvw}$	3.08^{vw}
	Festival	0.00^{w}	4.49^{uvw}	$10.20^{qrstuvw}$	13.12^{rstuvw}	9.04^{stuvw}
	Camarosa	0.00^{w}	10.51^{rstuvw}	6.45^{stuvw}	$18.72^{nopqrstuv}$	$14.77^{opqrstuvw}$
2	Fortuna	0.00^{w}	$14.15^{pqrstuvw}$	9.62^{stuvw}	$25.58^{klmnopqrs}$	9.87^{stuvw}
	Festival	0.00^{w}	$19.52^{mnopqrstuv}$	$18.23^{nopqrstuvw}$	$22.80^{lmnopqrst}$	$19.16^{nopqrstuv}$
	Camarosa	0.00^{w}	35.44^{klmn}	11.11^{rstuvw}	36.70^{klmn}	$17.68^{npqrstuvw}$
3	Fortuna	0.00^{w}	$21.74^{lmnopqrstu}$	10.89^{rstuvw}	35.60^{klmn}	$22.01^{lmnopqrstu}$
	Festival	0.00^{w}	$19.27^{nopqrstuv}$	30.74^{klmn}	44.06^{ghij}	34.95^{klmn}
	Camarosa	0.00^{w}	36.98^{jklmn}	$28.33^{klmnopqr}$	37.96^{jklm}	37.65^{jklm}
4	Fortuna	0.0^{w}	33.54^{klmn}	$30.77^{klmnopq}$	$54.44^{defghij}$	31.66^{klmnop}
	Festival	0.00^{w}	42.77^{ghij}	34.36^{klmn}	51.12^{efghij}	39.21^{ijkl}
	Camarosa	0.00^{w}	41.01^{ghij}	32.56^{klmno}	46.2^{fghij}	39.17^{ijkl}
5	Fortuna	0.00^{w}	$54.06^{defghij}$	57.10^{defghi}	70.97^{cd}	64.25^{def}
	Festival	0.00^{w}	52.17^{efghij}	40.62^{hijk}	57.78^{defgh}	$53.33^{defghij}$
	Camarosa	0.00^{w}	51.07^{fghij}	46.31^{ghij}	60.98^{defg}	43.98^{ghij}
6	Fortuna	0.00^{w}	86.66^{abc}	89.28^{ab}	100^{a}	80.45^{bcd}
	Festival	0.00^{w}	$53.33^{defghij}$	61.25^{defgh}	66.69^{cde}	44.16^{ghij}

Any two data in a table followed by the same letter are not statistically different at 5%

opment of vegetative and root biomasses of strawberry plants). The younger strawberries present abnormal growth and anthracnose symptoms appeared as brown necrotic tissues with round lesions of 2 to 3 mm covering fruits (Fig. 1A).

Symptom expression on leaves was characterized by brown spots, circular or oval in shape at the center and surrounded by a yellow halo of 1 to 2 mm (Fig. 1B). In advanced stages of lesion development, symptoms on leaves appear as dry, brown lesions develop along the margins and the leaves extremities (Fig. 1B). Then leaf blight and wilting were observed. Also, oval spots slightly depressed in the center and dark in color were formed on the petioles, peduncles and pedicels. The strong attacks of *Colletotrichum* isolates led to the leaves death and senescence of all strawberry plants in case of Fortuna variety (Fig. 1C and Fig. 3B).

In function of time, the progressive symptoms appearance expressed by evolution of severity index (Table 1) and infection coefficients (Table 2) of the tested *Colletotrichum* isolates on strawberry plants varieties was marked by a significant increase.

Seven days after inoculation, disease severity index and infection coefficients were low for all varieties and did not exceed 13.43% and 43.33, respectively. No statistical differences in severity index were found by the evaluation of all isolates in the varieties whereas the infection coefficient of Coll3 on Festival variety was significantly different from those of Coll1, Coll2 and Coll4. After four weeks, Coll3 exhibited the highest level of severity index and infection coefficients reaching respectively, 54.44% and 105 on Fortuna variety. A notable increase of virulence degree was checked in six weeks after

Table 2

Evolution of the infection coefficient (IC) in strawberry plants inoculated with *Colletotrichum* isolates according to time

Time (per week)	Varieties	Control	Coll1	Coll2	Coll3	Coll4
1	Camarosa	$0.00^{l'}$	$4^{j'k'l'}$	$0^{l'}$	$7.33^{h'i'j'k'l'}$	$0^{l'}$
	Fortuna	$0.00^{l'}$	$5.83^{i'j'k'l'}$	$3^{j'k'l'}$	$10^{f'g'h'i'j'k'l'}$	$1.6^{k'l'}$
	Festival	$0.00^{l'}$	$13.33^{f'g'h'i'j'k'l'}$	$16.5^{e'f'g'h'i'j'k'l'}$	$43.33^{xyza'}$	$18^{d'e'f'g'h'i'j'k'}$
2	Camarosa	$0.00^{l'}$	$24^{b'c'd'e'f'g'h'}$	$5.33^{i'j'k'l'}$	$35^{yza'b'c'd'}$	$8.33^{g'h'i'j'k'l'}$
	Fortuna	$0.00^{l'}$	$32.66^{za'b'c'd'e'}$	$25^{b'c'd'e'f'g'}$	48^{vwxyz}	$17.33^{e'f'g'h'i'j'k'l'}$
	Festival	$0.00^{l'}$	48^{vwxyz}	45.33^{wxyz}	65^{rstuv}	50^{uvwxyz}
3	Camarosa	$0.00^{l'}$	$26.66^{a'b'c'd'e'f'}$	$22.5^{c'd'e'f'g'h'i'}$	47.6^{vwxyz}	$20^{d'e'f'g'h'i'j'}$
	Fortuna	$0.00^{l'}$	55^{tuvwxy}	$21.66^{c'd'e'f'g'h'i'}$	62^{stuvw}	$38.66^{yza'b'c'}$
	Festival	$0.00^{l'}$	$14.16^{f'g'h'i'j'k'l'}$	$40.66^{xyza'b'}$	81.66^{nopq}	79.33^{opqrs}
4	Camarosa	$0.00^{l'}$	56.66^{tuvwx}	55^{tuvwxy}	99^{klmn}	75.83^{opqrs}
	Fortuna	$0.00^{l'}$	93^{lmno}	82.66^{nopq}	105^{ijklm}	66.73^{qrstu}
	Festival	$0.00^{l'}$	79.33^{opqrs}	$38.66^{yza'b'c'}$	85^{mnop}	51.66^{uvwxy}
5	Camarosa	$0.00^{l'}$	70.63^{pqrst}	$44^{xyza'}$	101.5^{jklm}	81.5^{nopqr}
	Fortuna	$0.00^{l'}$	125^{hi}	152^{g}	294^{c}	125.66^{hi}
	Festival	$0.00^{l'}$	90.66^{mno}	87.83^{mnop}	114^{ijk}	70^{qrst}
6	Camarosa	$0.00^{l'}$	135^{gh}	110^{ijkl}	187^{f}	115.5^{ijk}
	Fortuna	$0.00^{l'}$	378^{b}	300^{c}	408^{a}	198^{ef}
	Festival	$0.00^{l'}$	209.6^{de}	192.6^{def}	222^{d}	118.83^{hij}

Any two data in a table followed by the same letter are not statistically different at 5%

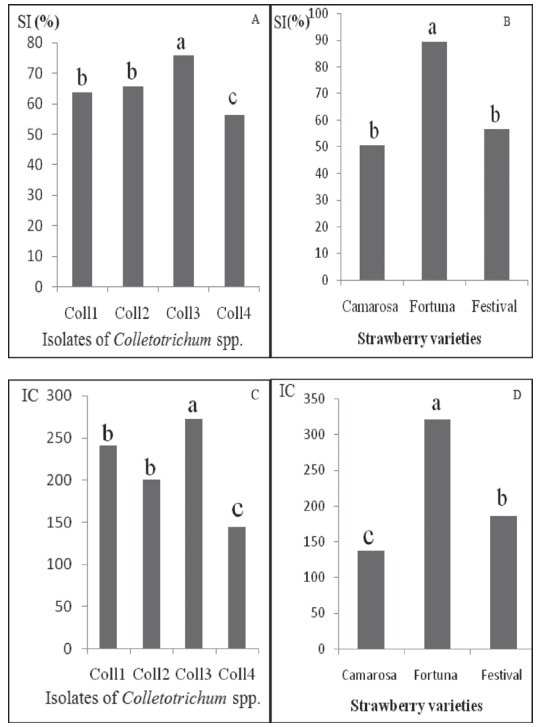

Fig. 2. Average percentage of severity index (IS) and infection coefficient (CI) in regard to *Colletotrichum* isolates (A and C) and the three strawberry varieties (B and D) after 6 weeks of inoculation

inoculation. In Fortuna variety inoculated with Coll3 isolate, the severity index and infection rates raised to 100% and 408, respectively, with significant differences between tested isolates. Coll4 presented the lowest severity index (43.98%) in Camarosa variety combined to infection coefficient of 115.5 slightly higher than that of Coll2 (110) but inferior to that of Coll1.

The results revealed that there were significant differences in disease severity between varieties. A various susceptibility levels were observed. Fortuna was the most susceptible strawberry variety to anthracnose showing higher severity index and infection coefficients rates (89.09% – 321) compared to Camarosa (Fig. 2B and D). Similarly, the comparison of severity index and infection coefficient values gathered at the sixth week, indicate the highest aggressiveness of *C. gleoesporioides* isolate (Coll3) which was the most pathogenic (75.89% – 272.33) (Fig. 2A and C). No symptoms were developed in the controls inoculated with sterile distilled water.

In regard of strawberry's infection, the percentage of rotten fruit had gradually increased by time. Seven days after inoculation, a weak deterioration was noted on Fortuna strawberries with the percentage of 6.66% and 8.33% in the presence of Coll2 and Coll3, respectively, while no symptoms were detected on varieties Camarosa and Festival. Four weeks after inoculation of the plants, the results revealed a significant difference between fruit reaction to isolates with a percentage of rotten fruit reaching 72.22% for Coll3 whereas those of the other isolates ranged between 30 and 37%. The final disease

level after six weeks of inoculation was more important. A maximal percentage of rotten strawberries was found on the strawberry plants inoculated with Coll3 isolate, 100% on Fortuna variety, followed by 83.33% on Festival variety and 70% on Camarosa variety but the lowest one was that of Coll1 on Festival variety (22.22%) (Table 3).

The measurement of the growth parameters of strawberry plants inoculated with *Colletotrichum* species revealed a notable reduction in leaf and root fresh and dry biomass. Thus, after six weeks of artificial inoculation (Fig. 3), the fresh weight of the aerial parts had significantly decreased compared to the control plants. In the presence of the Coll3 isolate, it was in the order of 9.96 g in Fortuna, 13.2 g in Festival and 22.76 g in Camarosa variety compared to 25 g, 35.8 g, 22.76 g and 34.4 g, respectively, in control plants. Likewise, the fresh biomass of the root system of the tested varieties inoculated by Coll3 was reduced nearly to half with respect to the control plants. It decreased, respectively, to 11.73 g (Fortuna); 18.53 g (Festival) and 19.36 g (Camarosa) (Table 4).

The inoculation tests had led to the reduction of the dry weights of both aerial parts and the root system of plants of the three strawberry varieties with different degrees. The Coll3 and the Coll4 isolates have caused a significant reduction of dry biomass of the aerial part of Fortuna and Festival varieties, respectively, equal to 1.86 g–3.886 g and

Table 3

Evolution in function of time of rotten strawberry's percentage six weeks after inoculation by *Colletotrichum* isolates

Time (per week)	Varieties	Control	Coll1	Coll2	Coll3	Coll4
1	Camarosa	0.00^{u}	0.00^{u}	0.00^{u}	0.00^{u}	0.00^{u}
	Fortuna	0.00^{u}	0.00^{u}	6.66^{tu}	8.33^{stu}	0.00^{u}
	Festival	0.00^{u}	0.00^{u}	0.00^{u}	0.00^{u}	0.00^{u}
2	Camarosa	0.00^{u}	0.00^{u}	0.00^{u}	$24.44^{klmnopqrst}$	$16.66^{klmnopqrst}$
	Fortuna	0.00^{u}	$30.66^{ghijklmnop}$	$18.33^{mnopqrstu}$	$36.66^{ghijklmno}$	$22.22^{lmnopqrstu}$
	Festival	0.00^{u}	11.11^{qrst}	$16.66^{nopqrstu}$	$14.28^{opqrstu}$	9.52^{rstu}
3	Camarosa	0.00^{u}	8.33stu	0.00^{u}	$24.99^{klmnopqrst}$	$20.50^{mnopqrstu}$
	Fortuna	0.00^{u}	$32.14^{hijklmnopqr}$	$20.11^{mnopqrstu}$	$32.22^{hijklmnopq}$	$26.36^{klmnopqrst}$
	Festival	0.00^{u}	14.24^{pqrstu}	$18.33^{mnopqrstu}$	$26.66^{jklmnopqrs}$	$26.38^{klmnopqrs}$
4	Camarosa	0.00^{u}	$16.66^{nopqrstu}$	$25.00^{klmnopqrst}$	60.91^{bcdef}	$44.44^{efghijkl}$
	Fortuna	0.00^{u}	$33.33^{hijklmnopq}$	$37.40^{ghijklmnop}$	72.22^{bc}	$30.28^{hijklmnopqrs}$
	Festival	0.00^{u}	$16.66^{nopqrstu}$	$28.45^{ijklmnopqrst}$	$40.36^{fghijklm}$	$28.22^{ijklmnopqrst}$
5	Camarosa	0.00^{u}	$20.14^{mnopqrstu}$	$27.3^{ijklmnopqrs}$	66.70^{bcde}	$47.16^{defghijk}$
	Fortuna	0.00^{u}	$35.40^{hijklmnop}$	$40.00^{fghijklmn}$	80.45^{ab}	$32.50^{hijklmnopq}$
	Festival	0.00^{u}	$20.4^{mnopqrstu}$	$39.15^{fghijklmno}$	$50.40^{cdefghi}$	$30.44^{hijklmnopqrs}$
6	Camarosa	0.00^{u}	$24.26^{klmnopqrst}$	$35.36^{hijklmnop}$	70.25^{bcd}	$50.00^{cdefghij}$
	Fortuna	0.00^{u}	60.00^{bcdefg}	52.38^{cdefgh}	100^{a}	$33.33^{hijklmnopq}$
	Festival	0.00^{u}	$22.22^{lmnopqrstu}$	$40.47^{fghijklm}$	83.33^{ab}	$44.77^{efghijkl}$

Any two data in a table followed by the same letter are not statistically different at 5%

Fig. 3. Aerial parts and root system growth of three strawberry varieties, (A) Camarosa, (B) Fortuna and (C) Festival, 6 weeks after inoculation with *Colletotrichum* isolates Coll1 (b), Coll2 (c), Coll3 (d) and Coll4 (e) compared to control plant (a)

3 g–3.76 g while those of control plant were in order of 14.9 g and 10.3 g. However, the dry weight of aerial parts of non-inoculated plants doubled those obtained in plants inoculated with Coll1 and Coll2. The aerial dry biomass of Camarosa variety inoculated with Coll3 (5.6 g) has decreased by half of that of control plants which showed a significantly identical dry biomass in the presence of the other isolates (Table 5). A considerable reduction of the dry weight biomass of the root system of strawberry plants was noted in case of inoculations by four tested *Colletotrichum* isolates (Table 5).

The re-isolation test was performed in order to describe whether the fungus invaded the plant. The colonization of *Colletotrichum* isolates of various plant organs of the strawberry varieties showed significant differences between the isolates (Table 6). The re-isolation percentage of Coll3 isolate from Fortuna variety leaves was positive and exceeded 93.33% followed by that of Coll2 (91.17%), and Coll1 (82.60%) whereas that of Coll4 was 46.66%. The re-isolation percentage of *Colletotrichum* isolates from leaves on other varieties was fluctuating varying from 41.66% to 61.53%.

Table 4

Effect of *Colletotrichum* isolates on Fresh weight of vegetative and root biomasses of strawberry plants six weeks after inoculation (expressed in g)

Isolates	Fresh weight					
	Aerial parts			Root parts		
	Cam	Frt	Fst	Cam	Frt	Fst
Control	34.40^{a}	25.00^{ab}	35.80^{a}	60.5^{a}	22.30^{cd}	42.80^{ab}
Coll1	24.66^{ab}	12.43^{bc}	19.03^{bc}	35.5^{bc}	18.53^{cd}	20.93^{cd}
Coll2	23.60^{abc}	15.93^{bc}	16.03^{bc}	22.9^{cd}	13.26^{d}	15.13^{d}
Coll3	22.76^{abc}	9.96^{c}	13.2^{bc}	19.36^{cd}	11.73^{d}	18.53^{cd}
Coll4	26.23^{ab}	10.53^{c}	14.43^{bc}	29.40^{bcd}	12.83^{d}	21.66^{cd}

Varieties: Cam: Camarosa; Frt: Fortuna; Fst: Festival

For each parameter, any two data in a table followed by the same letter are not statistically different at 5%

Table 5

Effect of *Colletotrichum* isolates on Dry weight of vegetative and root biomasses of strawberry plants six weeks after inoculation (expressed in g)

Isolates	Dry weight					
	Aerial parts			Root parts		
	Cam	Frt	Fst	Cam	Frt	Fst
Control	10.40^{b}	14.90^{a}	10.3^{b}	35.20^{a}	14^{bc}	19^{b}
Coll1	10.20^{b}	6.02^{cdef}	4.90^{cdefg}	13.50^{bc}	8.13^{cd}	6.6^{cd}
Coll2	8.13^{bcd}	7.10^{bcde}	4.46^{defg}	10.50^{bcd}	5.93^{cd}	6.2^{cd}
Coll3	5.60^{cdefg}	1.86^{g}	3.00^{fg}	6.63^{cd}	2.43^{d}	5.33^{cd}
Coll4	8.46^{bc}	3.86^{efg}	3.76^{efg}	14.26^{bc}	2.73^{d}	7.20^{cd}

Varieties: Cam: Camarosa; Frt: Fortuna; Fst: Festival

For each parameter, any two data in a table followed by the same letter are not statistically different at 5%

Table 6

Re-isolation frequencies (in %) of *Colletotrichum* from different organs of strawberry plants six weeks after inoculation

Isolates	Fortuna			Festival			Camarosa		
	Leaves	Crowns	Roots	Leaves	Crowns	Roots	Leaves	Crowns	Roots
Coll1	82.60^{a}	26.35^{cde}	0^{f}	53.33^{b}	13.33^{def}	0^{f}	44.44^{bc}	30.76^{cd}	0^{f}
Coll2	91.17^{a}	26.45^{cde}	0^{f}	56.25^{b}	7.69^{ef}	0^{f}	41.66^{bc}	28.57^{cd}	0^{f}
Coll3	93.33^{a}	53.33^{b}	0^{f}	58.33^{b}	26.66^{cde}	0^{f}	61.53^{b}	53.33^{b}	0^{f}
Coll4	46.66^{b}	26.66 ^{cde}	0^{f}	52.94^{b}	6.66^{ef}	0^{f}	42.85^{bc}	28.75^{cd}	0^{f}

Any two data in a table followed by the same letter are not statistically different at 5%

The highest re-isolation percentage of Coll3 isolate was obtained from Fortuna and Camarosa varieties crowns reaching 53.33% compared to 26.66% from Festival variety. A reduced re-isolation percentage of *C. acutatum* isolates (Coll1 and Coll2) were registered on Fortuna and Camarosa varieties crowns not exceeding 30.76% and superior to that noted on Festival crown (13.33%). However, no isolate was recovered from root system of all inoculated strawberry plants (Table 6).

Discussion and Conclusion

The pathogenicity of tested *Colletotrichum* isolates has been evidently demonstrated. The used varieties of strawberry plants inoculated with conidial suspension of *C. acutatum* and one isolate of C. *gloeosporioides* species showed the symptoms of anthracnosis on the aerial organs as well as a decrease of the vegetative and root biomass.

Indeed, the anthracnose, which is caused by the hemibiotroph *Colletotrichum* spp., is one of the most destructive diseases of cultivated strawberry (*Fragaria×ananassa* Duchesne) worldwide (Buddie et al., 1999; Münch et al., 2008; Debode et al., 2009). In fact, various symptoms are described on different strawberry plant parts such irregular and black leaf lesions, crown rot, flower blight and fruit rot (Freeman and Katan, 1997) considered as one of the most important pre-harvest fruit diseases caused by *C. acutatum* (Legard et al., 2003). In Manitoba, anthracnose fruit rot caused by *C. acutatum* appeared as small, irregular, tan or light brown, water-soaked lesions that dry, and mummified later (Xue and Davidson, 1995). In comparison, this disease produce a round, hollow, brownish, then black spots, covering with small orange spots, acervuli (Roger, 1953; Smith and Black, 1990). In agreement with our outcomes, an abnormal growth of strawberries was observed when early infection affects younger fruits (Roger, 1953). In addition, attacks can occur on all over the plant, including roots, leaves, flowers, runners, and fruits causing diseases such as defoliation, blossom blight and fruit rot (Bailey and Jeger, 1992). Additionally, in the present study *Colletotrichum* isolates have been recovered from crowns and roots of plants that exhibit a progressive wilt reaching whole plant. Similarly, according to Smith (1998), plants with symptoms of *Colletotrichum* crown rot show a sudden wilt of the entire plant and eventually the entire crown becomes discolored and the plant dies (Horn and Carver, 1963; Legard, 2000). As regards to *C. gloeosporioides* pathogenicity, Akhter et al. (2009) announced the development of a watery, irregular blotches and

pale brown spots 1 to 3 mm in diameter on strawberries, then tissues with lesions began to rot and/or blight when lesions enlarged and coalesced 6 to 8 days after inoculation. Howard and Albregts (1983) described a black leaf spot phase of strawberry caused by *C. gloeosporioides* which is often found in association with anthracnose symptoms on runners and petioles. In same way, the isolates of the two species tested in this study were able to damage both leaf tissues developing brown lesion in color that enlarged and dried as well as strawberries have been heavily affected by all isolates of the two species. Although, these species are pathogenic on strawberry, their survival and pathogenicity potential are not similar on the different organs of this plant. Thus, the study of the pathogenic ability of isolates originating from France showed the prevalence of *C. acutatum* on *C. gloeosporioides* that produced symptoms on the leaves of inoculated strawberry plants (Denoyes and Baudry, 1995). Whereas, other isolates of *C. gloeosporioides* have caused severe disease on strawberry leaves (Howard et al., 1992). Numerous studies have shown the ability of *C. gloeosporioides* to induce symptoms on blueberry leaves (Kim and Xiao, 2008), mango and piggyback plant (Pierce and McCain, 1990).

Based on our results of statistical evaluation, the capacity of *C. acutatum* isolates and *C. gloeosporioides* to infect the three varieties of strawberry plants was different. *C. gloeosporioides* was the most virulent and the Fortuna variety was more susceptible than the two others. Previous studies examining variation in strawberry resistance to *C. fragariae* or *C. gloeosporioides* found a broad range of susceptibility among cultivars and aggressiveness among isolates (Delp and Milholland, 1981; Smith and Black, 1990). Nowadays, there are no cultivars that exhibit complete resistance to *Colletotrichum* spp., thus aggravating its deleterious effects on strawberry production (Dodds and Rathjen, 2010; Amil-Ruiz et al., 2011). Many cultivated *Fragaria×ananassa* varieties were also characterized as the phenotypes resistant or tolerant to respective strawberry diseases in field conditions (Wing et al. 1995; Nelson et al., 1996; Bell et al., 1997; Shaw and Gordon 2003; Mori et al., 2005; Particka and Hancock, 2005; Zebrowska et al., 2006; Masny and Zurawicz, 2008). According to Seijo et al. (2008), different resistance levels were associated to strawberry cultivars currently grown in Florida. The variability of susceptibility could be related to some traits in plant. Among these, previous studies suggest that expression of resistance to *C. acutatum* disease may depend on the strain of the pathogen (Agostini et al., 1992; Chakraborty et al., 1997). Some strawberry cultivars have shown immunity to fruit rot caused by *C. acutatum* but more susceptibility to highly pathogenic isolates of *Colletotrichum* species that may cause a necrosis and crown rot (Delp and Milholland, 1981; Howard et al., 1992; Denoyes and Baudry, 1995; Freeman et al., 1997; Maas, 1998). The difference in resistance of strawberry cultivar to *C. acutatum* isolate seems to be polygenic (Denoyes and Baudry, 1995). However, the mechanisms underlying the genetic variation in the cultivated strawberry–*Colletotrichum* spp. interaction are largely obscure. According to Zhang et al. (2016), several PR genes were differentially expressed, with higher-amplitude changes observed in the less-susceptible cultivar which contained a higher level of basal salicylic acid defined as an extensive signaling role in plants, particularly in pathogen defense and which levels increased rapidly upon infection, followed by a sharp decrease before the necrotrophic phase. Curry et al. (2002) found that the invasion of strawberry variety tissues by *C. acutatum* and *C. fragariae* was similar; however, each invasion event occurred more rapidly with *C. fragariae* than with *C. acutatum* whose conidial and germ tube walls and appressorium appear to modify their chitin

distribution during initial contact with host tissue (O'Connell and Ride, 1990; Curry et al., 2002). The study of Wharton and Schilder (2008) demonstrated the ability of *C. acutatum* to adopt a different infection and colonization strategy depending on the susceptibility of the host tissue being colonized. In this context, results of histopathological study of Pardo et al. (2012) showed that tolerant species of strawberry to *C. gloeosporioides* present a noticeable accumulation of H_2O_2, a significant thickening of the cell wall in epidermal cells and changes of stomata and mesophyll cells whereas susceptible species did not and that the changes observed were correlated with the resistance to disease.

Moreover, the infection progress has led to significant adverse effects on normal growth of plants. Apparently, the inoculated strawberry plants placed in greenhouse and followed during 6 weeks showed a decrease of growth parameters as fresh and dry biomass of aerial parts and root system. According to Ndoutoume-Ndong (2007), the primary consequence of *Colletotrichum gloeosporioides* anthracnose on the rubber tree is the decrease in leaf area, leaf necrotic lesions and leaf fall thus reducing the plant's ability to synthesize organic matter following the decrease in photosynthesis. In accordance with our findings, the negative impact of *Colletotrichum* attack on plant growth parameters has also been visualized subsequent to potato roots infection leading to a poor plant emergence, growth, and early senescence (Dashwood et al., 1993). Also, tuber infection by *C. coccodes* producing severely affected seed yielded significantly less than healthy seeds (Read and Hide, 1995). Similarly, Nitzan et al. (2008) mentioned that plants grown from infected tubers had reduced yields even though they produced similar numbers of tubers as the non-inoculated plants.

In summary, the three varieties of strawberry plants were susceptible to *Colletotrichum acutatum* and *C. gloeosporioides* isolates responsible for anthracnose disease, among these varieties the Fortuna was the most susceptible. In addition, all isolates tested were able to cause symptoms on strawberry plants even though in varying degrees and reduce their agronomic parameters. It is necessary to think about using resistant varieties and to apply preventive and effective measures to prevent or reduce spread of these pathogens which constitute major threats not only for strawberry, but also for other economically important fruit crops, vegetables and fruits during pre- and postharvest period.

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